Biochem. J. (1990) 269, 1–11 (Printed in Great Britain)

REVIEW ARTICLE

The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life*

Paul J. THORNALLEY
Department of Chemistry and Biological Chemistry, University of Essex, Wivenhoe Park, Colchester, Essex CO4 3SQ, U.K.

INTRODUCTION

The glyoxalase system catalyses the conversion of methylglyoxal to D-lactic acid via the intermediate *S*-D-lactoylglutathione (Fig. 1). It comprises two enzymes, glyoxalase I and glyoxalase II, and a catalytic amount of reduced glutathione (Carrington & Douglas, 1986; Thornalley, 1989). Glyoxalase I (EC 4.4.1.5, lactoylglutathione lyase) catalyses the formation of *S*-D-lactoylglutathione from the hemithioacetal formed non-enzymically from methylglyoxal and reduced glutathione (Mannervik, 1980; Sellin *et al.*, 1983):

$$\underset{\text{Methylglyoxal}}{\text{MeCOCHO}} + \underset{\text{Reduced glutathione}}{\text{GSH}} \rightleftharpoons \underset{\text{Hemithioacetal}}{\text{MeCOCH(OH)-SG}} \xrightarrow{\text{Glyoxalase I}} \underset{\text{S-D-Lactoylglutathione}}{\text{MeCH(OH)CO-SG}}$$

Glyoxalase II (EC 3.1.2.6, hydroxyacylglutathione hydrolase) catalyses the hydrolysis of *S*-D-lactoylglutathione to D-lactic acid and regenerates the reduced glutathione consumed in the glyoxalase I-catalysed reaction (Uotila, 1989):

$$\text{MeCH(OH)CO-SG} + \text{H}_2\text{O} \xrightarrow{\text{Glyoxalase II}} \underset{\text{D-Lactic acid}}{\text{MeCH(OH)CO}_2\text{H}} + \text{GSH}$$

The glyoxalase system is present in the cytosol of cells and cellular organelles, particularly mitochondria. It is found throughout biological life and is thought to be ubiquitous (Carrington & Douglas, 1986). The widespread distribution and presence of the glyoxalase system in living organisms suggests it fulfils a function of fundamental importance to biological life. Yet, throughout nearly 80 years of study the biological function of glyoxalase has remained unclear.

Recent investigations have brought new developments in the involvement of the glyoxalase in cell growth and vesicle mobilization, with increasing evidence of changes in the glyoxalase system during tumour growth and diabetes mellitus, particularly relating to the development of associated clinical complications.

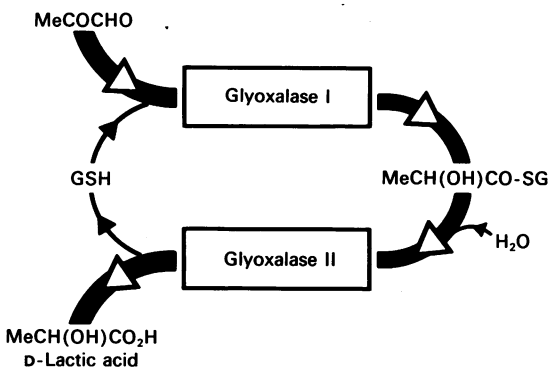


Fig. 1. The glyoxalase system

CELLULAR REGULATION OF THE GLYOXALASE SYSTEM

Regulation of the glyoxalase system in mammals

Cell proliferation and maturation. Glyoxalase phenotypes and enzymic activities have been studied during embryogenesis. Glyoxalase phenotypes were fully formed in aborted human foetuses between 6 and 7 weeks of gestational age (Wieczorek & Dobosz, 1979). They are among the earliest phenotypes expressed in embryogenesis. An active glyoxalase system is present through embryogenesis, tissue maturation and persists until cell death (McLellan & Thornalley, 1989). It appears crucial for life support.

Glyoxalase activities were studied in chicken liver during embryo development and after hatching. Tissue of immature embryo had a characteristic high activity of glyoxalase I and a low activity of glyoxalase II. Mature, differentiated tissue of chick liver 15 days after hatching had a characteristic low activity of glyoxalase I and a high activity of glyoxalase II (Principato *et al.*, 1982).

In regenerating rat liver after partial hepatectomy, glyoxalase I activity was significantly higher than sham-operated controls at 24 and 48 h after hepatectomy, later returning to control levels, and glyoxalase II activity was significantly lower at 24 h and higher after 48 and 72 h than sham-operated control, later returning to control levels (Principato *et al.*, 1983). Increased glyoxalase I activity with a similar temporal response was also found in partial hepatectomized liver by Alexander & Boyer (1971). Maximum mitosis and DNA synthesis occurs between 20 and 28 h after hepatectomy. Dixit *et al.* (1983) found a significant increase in DNA synthesis in partial hepatectomized liver in mice, maximizing at 24 h post-operation, with a corresponding temporal increase in the activity of glyoxalase I.

Irradiation of mouse spleen induced regeneration with associated increase in DNA synthesis and concomitant increase in the activity of glyoxalase I in spleen tissue. Colchicine (1 mg/kg) inhibited radiation-induced changes in DNA synthesis and glyoxalase I activity (Sutrave & Rao, 1982).

* This paper is dedicated to glyoxalase researchers everywhere.

These examples of regenerating and later maturing tissues suggest there are modifications of glyoxalase activities characteristic of the proliferation and differentiation status. Immature, proliferating tissue has a relatively high activity of glyoxalase I and low activity of glyoxalase II. Mature, differentiated tissues have relatively low activities of glyoxalase I and high activities of glyoxalase II. The activity of glyoxalase II may be more diagnostic and symptomatic since, generally, glyoxalase II is the rate-limiting enzyme in the glyoxalase system (Thornalley, 1989; Creighton *et al.*, 1988).

Vesicle mobilization. Functional stimulants of polymorphonuclear leukocytes, such as serum opsonised zymosan, chemotactic peptide *N*-formylmethionyl-leucyl-phenylalanine ('FMLP') and the tumour-promoting phorbol diester 12-*O*-tetradecanoylphorbol 13-acetate ('TPA') induce secretion of cytoplasmic granules from neutrophils (Klebanoff & Clark, 1978).

Phorbol diester induces secretion of specific granules from neutrophils, for which the receptor is thought to be protein kinase C. Stimulation of neutrophils with TPA induces an increase in the activity of glyoxalase I, a decrease in the activity of glyoxalase II (Gillespie, 1981; Thornalley & Bellavite, 1987) and a concomitant increase in cellular levels of *S*-D-lactoylglutathione (Thornalley & Bellavite, 1987). Changes in glyoxalase activities reflected changes in apparent maximum velocities ($V_{max.}$) of glyoxalase enzymes; K_m values were unchanged. Similar changes in glyoxalase activities and *S*-D-lactoylglutathione concentration were found in neutrophils stimulated with serum-opsonised zymosan (Thornalley *et al.*, 1987; Thornalley & Bellavite, 1987). *S*-D-Lactoylglutathione (10–20 μM) potentiates phorbol diester-stimulated secretion of specific granules from human neutrophils (5×10^6/ml) and also influences neutrophil movement; it increases random movement and inhibits chemotactic peptide-induced chemotaxis (Thornalley, 1989). Changes in glyoxalase activities and concomitant changes in cellular levels of *S*-D-lactoylglutathione may be involved in the regulation of degranulation (and possibly chemotaxis) in neutrophils.

A further example of vesicle mobilization is the secretion of histamine from basophils. Gillespie (1979) found that *S*-D-lactoylglutathione potentiated anti-IgE-induced secretion of histamine.

Regulation of the glyoxalase system in higher plants

The activity of glyoxalase I has been studied in several plants. In germinating pea, the activity of glyoxalase I was stimulated by indole acetic acid and inhibited by colchicine; it correlated with mitotic index (Ramaswamy *et al.*, 1983*a*). Glyoxalase I activity was also studied in *Datura callus* where it increased with increased growth, correlating with increase in DNA synthesis. Inhibitors of cell division, colchicine and vinblastine, arrested cell growth and decreased the activity of glyoxalase I. The glyoxalase I inhibitor squaric acid (4 mM) and high concentrations of methylglyoxal (> 10 mM) inhibited cell growth. Spermidine (1 μM) increased cell growth, protein and DNA synthesis, and glyoxalase I activity (Ramaswamy *et al.*, 1983*b*, 1984). Callus growth from leaf discs of *Brassica oleracea* was inhibited by trifluoroperazine (an inhibitor of calmodulin) and lithium chloride. There was a concomitant decrease in glyoxalase I activity with decreased cell growth (Bagga *et al.*, 1987).

Brassica cultures may be induced to undergo differentiation by lack of hormones in basal growth medium, and dedifferentiated by addition of 1-naphthaleneacetic acid and benzyladenine. The activity of glyoxalase I decreases by approx. 50% during differentiation (Sethi *et al.*, 1988). Glyoxalase II was not investigated in these studies.

Regulation of the glyoxalase system in micro-organisms

Yeast glyoxalase I activity is markedly affected by nutrient conditions. There was a 15–30-fold increase in glyoxalase I activity when yeast cells were grown on a medium containing glycerol as the carbon source (Penninckx *et al.*, 1983; Murata & Kimura, 1987) or L-threonine as the nitrogen source (Murata *et al.*, 1986*b*). The increase in glyoxalase I activity may be due to increased methylglyoxal formation from the catabolism of glycerol or L-threonine. The inability of glyoxalase I-deficient mutants to respond similarly and hence to grow in these media supports this hypothesis (Penninckx *et al.*, 1983; Murata *et al.*, 1986*b*). Glyoxalase I activity may also be induced by high concentrations of lactaldehyde (Murata & Kimura, 1987) and activity of glyoxalase II by hemithioacetal (Murata *et al.*, 1986*a*). TPA and mating factor induces an increase in glyoxalase I activity and a decrease in glyoxalase II activity in *Saccharomyces cerevisiae* (Murata & Kimura, 1987; Inoue *et al.*, 1989). Studies of *S. cerevisiae CDC* mutants have shown that the activity of glyoxalase I increases when yeast cells are grown on glucose as the carbon source and decreases in G_1-arrested cells (Dudani *et al.*, 1984; Murata & Kimura, 1986).

THE GLYOXALASE SYSTEM IN DISEASE PROCESSES

The glyoxalase system in tumour growth

The activity of glyoxalase I in tumour tissues is higher, lower or about the same as the activity in corresponding non-tumour tissues. However, the activity of glyoxalase II is consistently markedly lower in tumours than in corresponding normal tissues (Jerzykowski *et al.*, 1975*b*, 1978; Hooper *et al.*, 1987, 1988*a*,*b*; Thornalley & Bellavite, 1987; Thornalley, 1989).

Cell culture techniques have recently been used to study the modification of the human tumour cell glyoxalase system during drug-induced differentiation and serum deprivation-induced growth arrest. Hooper *et al.* (1987) investigated the changes in glyoxalase activities in human promyelocytic leukaemia 60 (HL60) cells and human erythroleukaemia K562 cells during drug-induced differentiation. For HL60 cells, there was a decrease in the activity of glyoxalase I and an increase in the activity of glyoxalase II during differentiation; the changes in glyoxalase activities were proportional to the extent of differentiation. For K562 cells, both activities of glyoxalase I and glyoxalase II increased during differentiation. During differentiation in K562 and HL60 cells, there was a decrease in the ratio of the activity of glyoxalase I to the activity of glyoxalase II during differentiation, with no significant change in the ratio during non-differentiating conditions.

The effect of changes in glyoxalase activities during maturation on the metabolism of methylglyoxal was assessed by determining the concentrations of methylglyoxal and *S*-D-lactoylglutathione, the rate of formation of D-lactate and the percentage of glucotriose (2 × rate of glucose consumption) metabolized to D-lactate during the differentiation of HL60 promyelocytes to neutrophil-like cells by *N*-methylformamide (Hooper *et al.*, 1988*a*). With the decrease in activity of glyoxalase I and the increase in activity of glyoxalase II accompanying differentiation, there were concomitant decreases in cellular methylglyoxal and *S*-D-lactoylglutathione concentrations, indicating that the activity of glyoxalase II has a major controlling influence over methylglyoxal and *S*-D-lactoylglutathione concentrations. There was also an increase in D-lactate formation.

The activity of glyoxalase II in HL60 cells is relatively low; hence, HL60 cells have a decreased capacity to metabolize *S*-D-lactoylglutathione. Incubation of HL60 cells with exogenous *S*-D-lactoylglutathione induces growth arrest, partial differentiation and toxicity. There is an accumulation of cells in G_0–G_1 of the cell cycle. The dose-dependence of cell cycle effects is consistent with cell death occurring at one phase of the cell cycle and decrease in the rate of progression of cells round the cell cycle by *S*-D-lactoylglutathione. The mechanism of this remarkable effect is presently unknown (Thornalley & Tisdale, 1988). Incubation of corresponding differentiated cells, neutrophils, with *S*-D-lactoylglutathione has no significant effect on cell viability in short-term culture (P. J. Thornalley, unpublished work).

To dissect modifications of glyoxalase activity relating to changes in the cell cycle or to changes in differentiation status, two cell lines were studied, human transformed lymphoblastoid GM892 cells and Burkitt's lymphoma Raji(+) cells, which could be growth arrested by serum deprivation without inducing differentiation. Growth-arrested GM892 cells had a decreased activity of glyoxalase I and growth-arrested Raji cells had an increased activity of glyoxalase I relative to rapidly proliferating cells. However, the activity of glyoxalase II, which was rate-limiting in the glyoxalase system of both cell lines, increased during growth-arrest in both cell lines. This corresponded to an increase in the percentage of cells in the G_0–G_1 phase of the cell cycle in growth arrest. This suggests that glyoxalase activities change round the mammalian cell cycle and that the changes in glyoxalase activities during drug-induced differentiation may be, in part, cell cycle-related (Hooper *et al.*, 1988*b*).

The activity of glyoxalase II in cells consistently increases during growth arrest and differentiation. Immature, rapidly proliferating tissues have a relatively low capacity to metabolize *S*-D-lactoylglutathione and are particularly susceptible to toxicity of this compound.

Modification of the glyoxalase system in diabetes mellitus

When human red blood cells are incubated in short-term culture with 5–50 mM-glucose *in vitro*, the flux of methylglyoxal metabolized to D-lactate via the glyoxalase system increases. There is no change in glyoxalase activities but the increased flux produces increased cellular concentrations of methylglyoxal and *S*-D-lactoylglutathione (Thornalley, 1988). Red blood cells have insulin-independent glucose uptake and, hence, experience periodic hyperglycaemia during diabetes mellitus, the cytoplasmic glucose concentration reflecting the rise and fall of plasma glucose levels during management of the disease. Methylglyoxal formation is probably increased by the increased production of dihydroxyacetone phosphate in the Embden–Meyerhof pathway and from fructose metabolism produced from the polyol pathway (Taylor & Agius, 1988).

Modification of the glyoxalase system in red blood cells during experimental diabetes mellitus was investigated in streptozotocin-induced diabetic mice (a model for insulin-dependent diabetes mellitus) and in obese (*ob*/*ob*) mice (a model for non-insulin-dependent diabetes mellitus). In both models, during the onset of diabetes, the activity of red blood cell glyoxalase I decreased by 50–60% of control values, and the activity of red blood cell glyoxalase increased by 20–30% of control values. The whole blood concentration of methylglyoxal increased but the whole blood concentration of *S*-D-lactoylglutathione was not significantly changed in both diabetes models (Thornalley & Atkins, 1989). Changes in glyoxalase activities did not correspond to those expected for a change in age distribution of red blood cells (McLellan & Thornalley, 1989). It is clear that onset of diabetes either influences glyoxalase synthesis in red blood cell precursors or induces a post-translational modification of glyoxalase in red blood cells.

The effect of clinical diabetes mellitus on the red blood cell glyoxalase system has been assessed in patients with insulin-dependent and non-insulin-dependent diabetes mellitus and normal healthy controls. In both diabetic types, the concentrations of methylglyoxal and *S*-D-lactoylglutathione in blood samples were significantly elevated. For insulin-dependent diabetics, matched for age and duration of disease, patients with clinical complications had a significantly higher activity of glyoxalase I and a significantly lower activity of glyoxalase II than uncomplicated patients. Taken together, this suggests that patients with complications had a propensity to maintain relatively high levels of *S*-D-lactoylglutathione (Thornalley *et al.*, 1989).

BIOLOGICAL FUNCTION OF THE GLYOXALASE SYSTEM

Although it has been known for many years that the glyoxalase sytem catalyses the conversion of methylglyoxal to D-lactate, the biological function of this process has remained unclear. Historically, investigations have been directed towards the biological effects of methylglyoxal to resolve this problem.

A BIOLOGICAL FUNCTION ASSOCIATED WITH THE METABOLISM OF METHYLGLYOXAL

When Dakin & Dudley (1913) and, working independently, Neuberg (1913) discovered the glyoxalase system, it was thought that it represented a major metabolic pathway for the conversion of glucose to L-lactate. Following the discovery of phosphorylated glycolytic intermediates, this was discounted. Hopkins & Morgan (1945) studied the widespread distribution of glyoxalase activity throughout biological life and recognized that this betrayed an important cellular function.

Later, Racker (1951, 1954) established that the glyoxalase system metabolized methylglyoxal to D-lactate via the intermediate *S*-D-lactoylglutathione. This fundamentally divorced the glyoxalase system from mainstream glycolysis, known to produce L-lactate. Meantime, the biological effects of methylglyoxal, particularly relating to cytostatic activity, were being investigated.

Biological formation of methylglyoxal

Undoubtedly, the greatest blight on our understanding of the presence (and function) of methylglyoxal in biological systems is the poor performance of assay techniques deployed in its measurement. A review has recently appeared on this subject (Ohmori *et al.*, 1989).

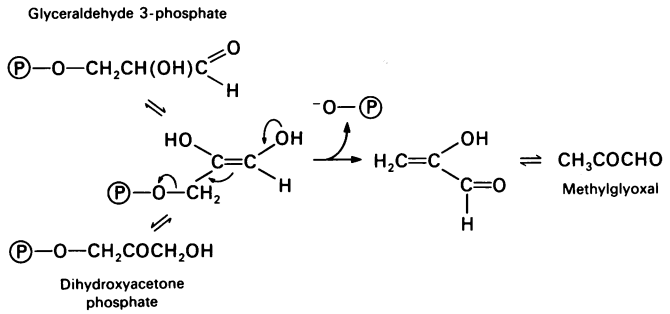
Traditionally, methylglyoxal was determined by reaction with 2,4-dinitrophenylhydrazine, with or without chromatographic separation of the resultant dihydrazone (osazone) and spectrophotometric detection (Vogt, 1929; Fung & Grosjean, 1981). This was used by Fodor *et al.* (1978) and Sato *et al.* (1980) to demonstrate the presence of methylglyoxal in liver and by Riddle & Lorenz (1968) to measure non-enzymic formation of methylglyoxal from glyceraldehyde. It now appears that 2,4-dinitrophenylhydrazine will form the same osazone by direct reaction with glyceraldehyde, glyceraldehyde 3-phosphate, dihydroxyacetone and dihydroxyacetone phosphate under acidic conditions (Ohmori *et al.*, 1989). Under the conditions used by Riddle & Lorenz (1968), glyceraldehyde can oxidize to hydroxypyruvaldehyde which can also form an osazone (Thornalley *et al.*, 1984; Thornalley, 1985, 1986). The reliability of estimates of the

formation of methylglyoxal in biological systems by the 2,4-dinitrophenylhydrazine method is questionable.

The interferences in assays of methylglyoxal have maintained a persistent controversy as to the presence and enzymic or non-enzymic formation of methylglyoxal in biological systems; this should be kept in mind when considering reviews of the older literature (e.g. Schauenstein *et al.*, 1977). The prospect for resolution of this problem resides in more specific and reliable assay techniques recently reported.

Assays for methylglyoxal involving derivatization with *o*-phenylenediamine to 2-methylquinoxaline (Ohmori *et al.*, 1987*a*; Thornalley, 1988) and with 4,5-dichloro-1,2-phenylenediamine to 6,7-dichloro-2-methylquinoxaline (Ohmori *et al.*, 1987*b*), have been developed. These methods appear not to be subject to the same or similar interferences as the 2,4-dinitrophenylhydrazine method (Ohmori *et al.*, 1989). Using these methods, biological sources of methylglyoxal have been investigated.

From recent studies, the main source of methylglyoxal in mammals appears to be glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Formation occurs by base-catalysed phosphate elimination, probably from the small amount of endiol form of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in solution (Ohmori *et al.*, 1989), as previously indicated by Richard (1985).



At present, it is not clear if there is significant enzymic formation of methylglyoxal in mammalian systems; the *o*-phenylenediamine-based methylglyoxal assay procedures have yet to be applied to methylglyoxal synthase-catalysed reactions. Methylglyoxal formation appears to be an intrinsic feature of the Embden–Meyerhof pathway, a corollary to the presence of triose-1,2-enediol 3-phosphate in solution at physiological pH. However, only approx. 0.1–0.4% of glucotriose was metabolized to D-lactate in human red blood cells and promyelocytic leukaemia cells (Thornalley, 1988; Hooper *et al.*, 1988*a*) which represents the maximum flux of methylglyoxal formation, with subsequent conversion to D-lactate by the glyoxalase system. So, although apparently an unavoidable consequence of the phosphotriose economy in cells, methylglyoxal formation and metabolism normally represent only a very minor fate of glucotriose.

Other sources of methylglyoxal in mammalian metabolism are: (i) from the oxidation of acetol in the metabolism of acetone (Casazza *et al.*, 1984; Reichard *et al.*, 1986), and (ii) from aminoacetone formed in threonine catabolism by amine oxidase (Ray & Ray, 1983, 1987). However, under normal metabolic control, these appear to be minor sources of methylglyoxal (Ohmori *et al.*, 1989).

In prokaryotic micro-organisms, methylglyoxal is formed non-enzymically from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate and also enzymically from dihydroxyacetone phosphate catalysed by methylglyoxal synthase (Cooper, 1984). Methylglyoxal synthase has been purified from *Escherichia coli*, and purified and crystallized from *Proteus vulgaris* (Hopper & Cooper, 1972; Tsai & Gracy, 1976). The enzyme from *E. coli* has a molecular mass of 67 kDa and that from *P. vulgaris* 135 kDa. The enzyme is inhibited by physiological levels of inorganic phosphate, P_i. Its consequent activation when P_i levels fall and initiation of the conversion of dihydroxyacetone phosphate to lactate (via the glyoxalase system), liberating P_i, is particularly important in the microbial metabolism of glycerol where triose phosphate intermediates may otherwise accumulate to toxic levels (Cooper, 1984; Penninckx *et al.*, 1983).

Reaction of methylglyoxal with biological molecules

Amino acids and proteins. Methylglyoxal reacts with arginine and arginyl residues in proteins (Takahashi, 1977*a*,*b*; Cheung & Fonda, 1979). Methylglyoxal (> 10 mM) inhibits glycolytic enzymes (Leonici *et al.*, 1989) and inhibits microtubule assembly (Miglietta & Gabriel, 1986). Since estimates of the physiological concentration of methylglyoxal are in the range 0.1–2 μM (Ohmori *et al.*, 1987*b*; Thornalley *et al.*, 1988), these effects on protein function are expected to be of little physiological significance.

Reaction of methylglyoxal with nucleic acids, RNA and DNA. The reactivity of methylglyoxal with nucleic acids was investigated by studying radiolabelling of polynucleotides by ^{14}C-labelled methylglyoxal. Methylglyoxal binds to polyguanosine preferentially: relative binding to polyguanosine, adenine, cytosine and urosine was 100:7:3:0 (Shapiro *et al.*, 1969; Krymkiewicz, 1973). Methylglyoxal also binds reversibly to tRNA, but only little binding was observed to DNA. Binding to DNA was increased markedly by prior heat denaturation. It is a weak inhibitor of translation of natural and chemically decapped mRNA (Kozarich & Deegan, 1979; Lozano & Mezl, 1984) and induced crosslinks in DNA, as judged by the alkaline elution technique (Marinari *et al.*, 1984). DNA crosslinking was observed with 1.5 mM-methylglyoxal in Chinese hamster ovary cells. Crosslinks appeared to be between DNA and protein and were fully repaired after 24 h (Brambilla *et al.*, 1985). Methylglyoxal produces an increase in sister-chromatid exchanges, which correlates to mutagenic/carcinogenic activity (Faggin *et al.*, 1985). Again, these effects may be of little physiological significance because they are observed at methylglyoxal concentrations 100–1000 times that found physiologically.

Biological effects of methylglyoxal

The late Albert Szent-Gyorgyi and co-workers were among the first groups to become interested in the biological effects of methylglyoxal. Cultures of *E. coli* were incubated with 0.2–1.0 mM-methylglyoxal. There was a reversible inhibition of

cell growth with 1 mM-methylglyoxal, the most effective concentration inhibiting growth from 6 h (Egyud & Szent-Gyorgyi, 1966*a*). This was associated with the inhibition of protein synthesis (Egyud & Szent-Gyorgyi, 1966*b*). A more recent study shows that methylglyoxal (0.25 mM) inhibits the growth of asynchronous *E. coli* cells (3.7×10^6/ml) in culture but higher concentrations are required to inhibit growth for higher cell densities (Fraval & McBrien, 1980). Szent-Gyorgyi and others suggested methylglyoxal was a physiological growth-inhibiting substance, and with its biological antagonist glyoxalase I, it controlled cell growth. Recent estimates of cellular concentrations of methylglyoxal, 0.1–2 μM (Ohmori *et al.*, 1987*b*; Thornalley *et al.*, 1988) have suggested that this property of methylglyoxal may be of limited physiological significance. However, there was still a possibility that the growth inhibition induced by high concentrations of methylglyoxal might be pharmacologically useful in cancer chemotherapy.

Ascites tumours in albino mice were susceptible to the cytostatic effects of methylglyoxal at concentrations > 1.0 mM (Egyud & Szent-Gyorgyi, 1968). French & Freelander (1958) had earlier reported that methylglyoxal (500 mg/kg) increased the mean lifetime of mice with ascites L1210 leukaemia by 31% (see below).

An extensive study of the effect of methylglyoxal on tumour growth was reported by Apple & Greenberg (1967). Mice were innoculated with tumour cells and given intraperitoneal injections of methylglyoxal (70–80 mg/kg daily). Growth inhibition was 90–99% for a range of ascites tumours. A single dose of 215–240 mg/kg intraperitoneally also inhibited tumour growth by 80–99%. Similar inhibition of growth of Ehrlich ascites carcinoma in mice, Yoshida ascites carcinoma in rats and Kirkman–Robbins hepatoma in hamsters by methylglyoxal was reported when administered by intraperitoneal injection (75–175 mg/kg) for ascites tumours and subcutaneous injection (11–110 mg/kg) close to the solid tumour. Methylglyoxal was relatively ineffective against L-1210 leukaemia (Jerzykowski *et al.*, 1970).

Given these data, it is perhaps surprising that methylglyoxal therapy is not widely used in cancer chemotherapy. The reason for this is that methylglyoxal is ineffective *in vivo* when administered other than peritumorally. Moreover, even for such instances, as for intraperitoneal injection for ascites tumours, doses effective for growth inhibition impair respiration and cardiac function (Jerzykowski *et al.*, 1975*a*; Conroy, 1979). Some investigators have suggested that the reason for lack of a potent antitumour effect of methylglyoxal is due to its rapid metabolism by glyoxalase and other enzyme systems. However, the toxicological assessment of methylglyoxal indicates that even if methylglyoxal levels could be elevated by a high dose or continuous infusion of methylglyoxal, or by inhibition of glyoxalase I, the corresponding impairment of cardiac and respiratory function would prevent exploitation of the antitumour effects (Jerzykowski *et al.*, 1975*a*).

The recognition of the toxicity of methylglyoxal has prompted the proposal that the function of the glyoxalase system is the detoxification of methylglyoxal. Mannervik and co-workers suggested the function of the glyoxalase system was to protect mammals from methylglyoxal produced by intestinal microflora (Mannervik, 1980). Indeed, there is experimental evidence to support a detoxification function: an *E. coli* clone with a 200-fold increase in activity of glyoxalase I was resistant to the toxicity of a challenge of 5 mM-methylglyoxal (Rhee *et al.*, 1987), and a *Saccharomyces cerevisiae* mutant deficient in glyoxalase I activity was particularly sensitive to methylglyoxal toxicity (Penninckx *et al.*, 1983). Yet, in these examples, the argument for the glyoxalase system functioning as a detoxification system is clear because the threat to cell survival is clear under these experimental conditions. In normal, physiologically functioning cell systems, the concentration of methylglyoxal is low (0.1–2 μM; Ohmori *et al.*, 1987*b*; Thornalley *et al.*, 1988) and the rate of formation of methylglyoxal is low (the flux through the glyoxalase system amounts for only 0.08–0.40% of glucotriose metabolized; Thornalley, 1988; Hooper *et al.*, 1988). It is not clear if methylglyoxal is a viable concept under physiological conditions. Moreover, the regulation of the activities of glyoxalase enzymes during cell proliferation, differentiation and vesicle mobilization by functional stimulants and during onset of experimental diabetes suggests an, as yet, undisclosed role in cellular function for *S*-D-lactoylglutathione and glyoxalase enzymes.

A BIOLOGICAL FUNCTION ASSOCIATED WITH GLYOXALASE ENZYMES AS REGULATORS OF *S*-D-LACTOYLGLUTATHIONE CONCENTRATION

Much is now known on the properties of glyoxalase enzymes.

Glyoxalase I

Distribution. Glyoxalase I activity is present in all tissues of prokaryotic and eukaryotic organisms. It has been detected in: mammals, birds, fish, amphibians, plants, bacteria, fungi, protozoans and invertebrates (Jeryzkowski *et al.*, 1978; Hopkins & Morgan, 1945; Darling & Blum, 1988). Immunoassay of glyoxalase I has shown the presence of approx. 0.2 μg/mg of protein in most human tissue (Larsen *et al.*, 1985).

Molecular characteristics (Table 1). Mammalian glyoxalase I has a molecular mass of 43–48 kDa and is a dimer of two identical or similar subunits. Glyoxalase I from prokaryotic and eukaryotic micro-organisms is a monomer of 20–36 kDa. Yeast glyoxalase I is thought to contain 0.75% (w/v) carbohydrate (Douglas *et al.*, 1986). Each subunit of dimeric mammalian glyoxalase I and each monomer of microbial glyoxalase I contains one zinc ion, Zn^{2+}. The apoenzyme is catalytically inactive. Metal ion substitution experiments have demonstrated that similar specific enzymic activities can be maintained with Mg^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} ions (Han *et al.*, 1977; Sellin *et al.*, 1983). Mammalian glyoxalase I has an isoelectric point of 4.7–4.8, an acidic protein, whereas the yeast enzyme has an isoelectric point of 7.0 (Marmstal & Mannervik, 1978; Marmstal *et al.*, 1979). Antibodies raised against mammalian glyoxalase I will cross-react with antigen from other mammalian species, but not with antigen from yeast (Larsen *et al.*, 1985).

Substrate specificity. The physiological cofactor for glyoxalase I is reduced glutathione (γ-L-glutamyl-L-cysteinylglycine). L-Cysteine, oxidized glutathione, L-cysteinylglycine and γ-L-glutamylcysteine are inactive as cofactors (Carnegie, 1963; Wieland *et al.*, 1956*a*; Behrens, 1941).

Glyoxalase I has a broad substrate specificity for α-oxoaldehydes. Substrates include methylglyoxal, hydroxypyruvaldehyde, hydroxypyruvaldehyde phosphate, glyoxal, phenylglyoxal and many other alkyl- and aryl- glyoxals (Reeves & Ajl, 1965; Weaver & Lardy, 1961; Vander Jagt *et al.*, 1975; Carrington & Douglas, 1988). The K_m and $V_{max.}$ decrease as the hydrophobicity of the side chain increases.

Kinetics. The molecular species which binds to the active site of glyoxalase I is the hemithioacetal formed non-enzymically from α-oxoaldehyde and reduced glutathione (Marmstal & Mannervik, 1981). The predominant physiological α-oxoaldehyde involved in this reaction is methylglyoxal. The hemithioacetal is formed from the unhydrated form of methyl-

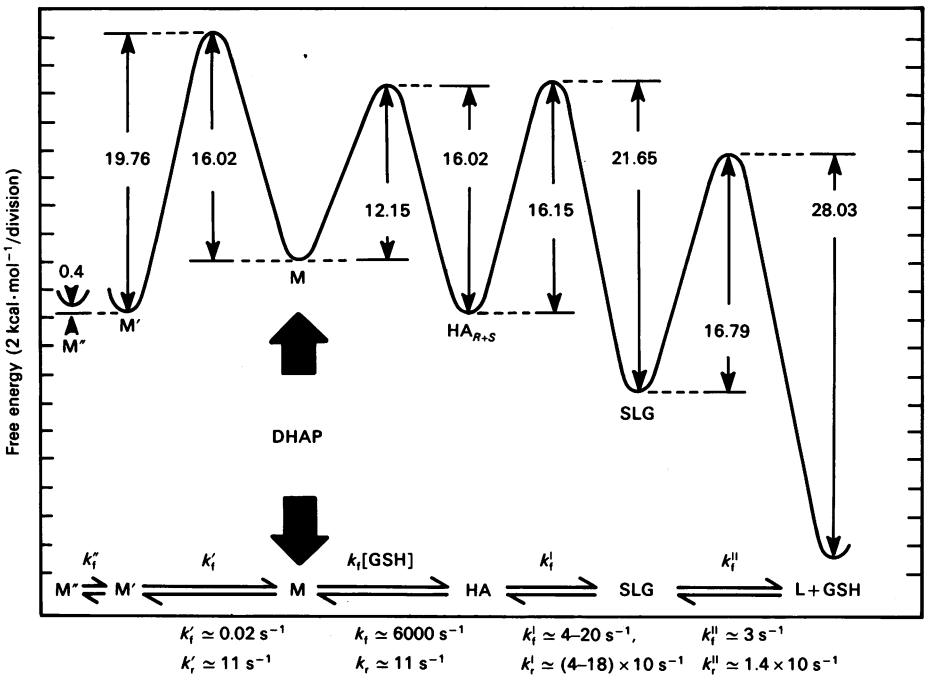


Fig. 2. Equilibria for the interconversion of the hydrated forms of methylglyoxal and the glyoxalase system

Free energy diagram for the red blood cell glyoxalase system. (Data adapted from Creighton *et al.*, 1988.) Abbreviations: M, methylglyoxal; HA, hemithioacetal; SLG, *S*-D-lactoylglutathione; L, D-lactate; DHAP, dihydroxyacetone phosphate.

glyoxal. In aqueous solution at pH 7, methylglyoxal exists as α-oxoaldehyde, monohydrate and dihydrate forms (Fig. 2).

$$\underset{\text{Dihydrate}}{MeC(OH)_2CH(OH)_2} \overset{-H_2O}{\underset{+H_2O}{\rightleftharpoons}} \underset{\text{Monohydrate}}{MeCOCH(OH)_2} \overset{-H_2O}{\underset{+H_2O}{\rightleftharpoons}} \underset{\alpha\text{-Oxoaldehyde}}{MeCOCHO} \overset{+GSH}{\underset{-GSH}{\rightleftharpoons}} \underset{\text{Hemithioacetal}}{MeCOCH(OH)\text{-}SG}$$

Under these conditions, in the presence of 2 mM reduced glutathione (as is found in red blood cells), 41 % of methylglyoxal exists as the hemithioacetal and only 0.04 % of methylglyoxal exists in the α-oxoaldehyde form implicated in protein cross-linking and binding of nucleic acid. Under physiological conditions, methylglyoxal may be produced as the α-oxoaldehyde form which reacts rapidly with reduced glutathione avoiding routes via the hydrated forms. The high reactivity of unhydrated methylglyoxal formed *in situ* has been recognized by Patthy (1978).

Glyoxalase I catalyses the isomerization of the hemithioacetal to *S*-D-lactoylglutathione. The rate approximates to Michaelis–Menten kinetics over a restricted substrate concentration range below saturation conditions. The initial velocity–saturation behaviour is non-Michaelian. With purified enzymes, Mannervik found evidence for competitive inhibition by glutathione at high concentration (Marmstal & Mannervik, 1981). The Michaelis constant, K_m, values for the hemithioacetal with purified enzyme are given in Table 1. The molecular activity (turnover number), $k_{cat.}$, is (59–71) × 10^3 min^{-1} for mammalian enzymes and 35400 min^{-1} for the yeast enzyme; the molecular activities per subunit for the enzymes are similar. The values of $k_{cat.}/K_m$ are 10^7–10^8 $M^{-1} \cdot s^{-1}$, which is close to the theoretical diffusion limit maximum, indicating that glyoxalase I is a very efficient enzyme (Marmstal *et al.*, 1979).

Studies of the active site. Chemical modification studies of mammalian glyoxalase I suggest there is a tyrosyl residue, a tryptophanyl residue, a lysyl residue and possibly a glutamyl or aspartyl residue in the active site of glyoxalase I (Aronsson *et al.*, 1981; Mannervik *et al.*, 1975; Baskaran & Balasubramanian, 1987). In contrast, yeast glyoxalase I has a cysteinyl residue, an arginyl residue and a tyrosyl residue in the active site (Ekwall & Mannervik, 1970; Schasteen & Reed, 1983; Carrington & Douglas, 1985).

Catalytic mechanism of glyoxalase I. The mechanism proposed for the glyoxalase I reaction consistent with experimental evidence involves a shielded-proton transfer from C-1 to C-2 of the hemithioacetal, bound in the active site, to form an ene-diolate intermediate and rapid ketonization to the thioester product (Fig. 3).

The identity of the base, B, which deprotonizes the hemithioacetal, is uncertain. It has been suggested that a histidine residue fulfils this role as a general base catalyst. The ability of imidazole to promote the analogous non-enzymic reaction suggests this is possible, yet, the evidence for a histidine residue in the active site of glyoxalase I is presently not widely accepted (Hall *et al.*, 1978; Douglas, 1987).

Genetics and polymorphism. Human glyoxalase I was found to exhibit genetic polymorphism originally in red blood cells (Kompf *et al.*, 1975*a*). There are three phenotypes, GLO 1-1, GLO 2-1 and GLO 2-2, representing the homozygous and heterozygous expression of a two-allelic gene, GLO^1 and GLO^2 at an autosomal locus. GLO 1-1 is electrophoretically 'slow' and GLO 2-2 is electrophoretically 'fast' (Kompf *et al.*, 1975*b*). An analogous phenotypic expression has been found in 23 different

Table 1. Molecular properties of glyoxalase I

References: Marmstal *et al.* (1979), Aronsson & Mannervik (1977), Han *et al.* (1976), Marmstal & Mannervik (1979), Oray & Norton (1980), Baskaran & Balasubramanian (1987), Rhee *et al.* (1986), Douglas *et al.* (1986).

Source	Molecular mass (kDa)	No. of subunits	Isoelectric point (pH)	Kinetic constants for methylglyoxal	
				K_m (μM)	$k_{cat.}$ (min^{-1})
Human erythrocyte	46	2	4.8	130	6.8×10^4
Monkey intestinal mucosa	48	2	–	500	–
Pig erythrocyte	48	2	4.8	190	–
Rat liver	46	2	4.7	140	–
Sheep liver	46	2	5.0	78	–
Mouse liver	43	2	–	–	–
Yeast (*Saccharomyces cerevisiae*)	32	1	7.0	512	3.54×10^4
Bacteria (*Escherichia coli*)	34	1	–	–	–
(*Rhodospirillum*)	31	1	–	–	–
(*Pseudomonas putida*)	20	1	4.0	3500	–
Mould (*Aspergillus niger*)	36	1	–	1250	–

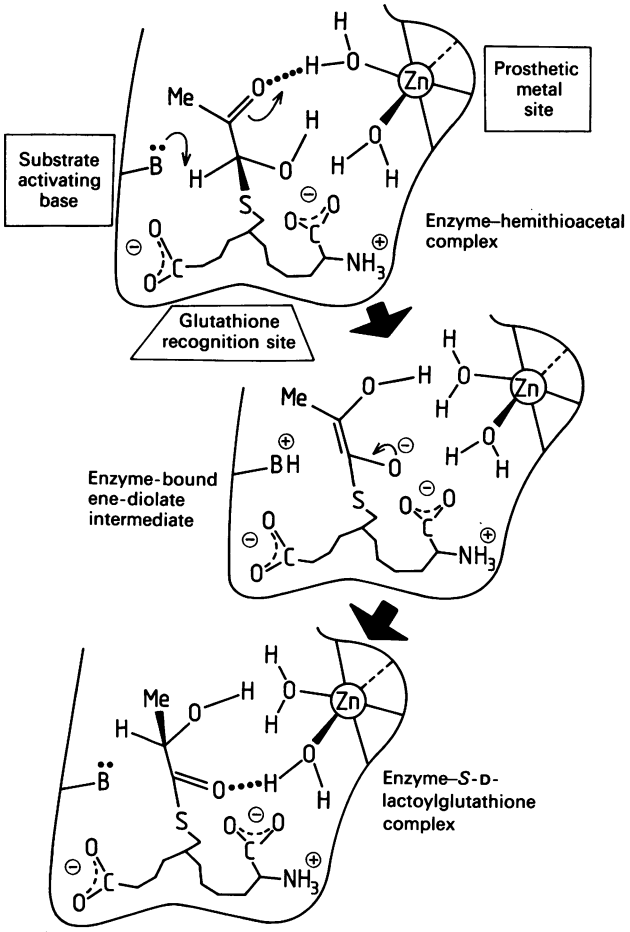


Fig. 3. Mechanism for the glyoxalase I-catalysed isomerization of hemithioacetal to *S*-D-lactoylglutathione

(Sellin *et al.*, 1983.)

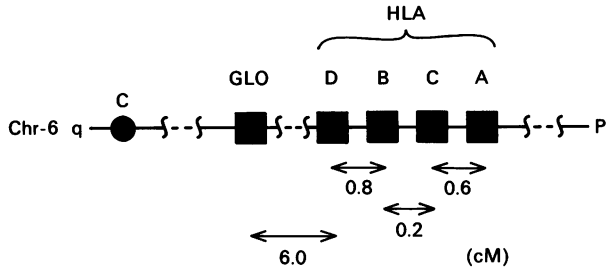


Fig. 4. Genetic locus of the gene for human glyoxalase I

GLO and the HLA complex are located on chromosome 6 (Bakker *et al.*, 1979).

human tissues in each of 49 autopsies (Stohlmacher & Haferland, 1980).

The GLO^1 and GLO^2 alleles are inherited autosomally in a two-allele codominant manner and characteristic phenotypic expressions are present in all tissues. The GLO locus has been assigned to chromosome 6 (Bender & Grzeschik, 1976), close to the major histocompatibility complex (Kompf *et al.*, 1976). GLO is between the centromere and HLA-DR (Bakker *et al.*, 1979; Leach *et al.*, 1986); the meiotic distance between GLO and HLA-DR loci is ~ 6 cM (Fig. 4). It is not clear why major histocompatibility antigens and GLO loci have remained linked through evolution in spite of a relatively large recombination distance.

The GLO genetic locus has been linked to insulin-dependent diabetes mellitus. A study of GLO gene frequencies has shown that there is a significant excess of glyoxalase homozygote GLO 1-1 and a deficiency of types GLO 2-1 and GLO 2-2 in insulin-dependent diabetic patients relative to controls (McCann *et al.*, 1981). A GLO^1 allele frequency in insulin-dependent diabetes higher than in non-diabetic patients was reported by Cambdon-de Mouzon *et al.* (1982). Other studies have not found changes in GLO 1-1 phenotype or GLO^1 allele frequency in insulin-dependent diabetic patients (Tokanaga *et al.*, 1982; Kirk *et al.*, 1985). For non-insulin-dependent diabetic patients, Kirk *et al.* (1979) found an excess of GLO 2-2 phenotype and a deficiency of GLO 1-2 phenotype whereas other surveys (Kirk *et al.*, 1985; McCann *et al.*, 1981) produced no significant difference. However, more interestingly, insulin-dependent diabetic patients without clinical complications had a significant excess of the homozygote GLO 1-1 whereas patients with complications did not (McCann *et al.*, 1981). Our experimental results have suggested that there is a correlation between glyoxalase activities and the development of clinical complications in insulin-

dependent diabetic patients. The work of McCann *et al.* (1981) suggests there may be a genetic trait for resistance to diabetic complications. These studies hold great promise for the future.

Inhibition of glyoxalase I. Inhibitors of glyoxalase I have been designed from analogues of the hemithioacetal substrate based on glutathione derivatives and analogues of the enediol moiety of the putative transition state. Other inhibitors have been discovered by serendipity (Jordan *et al.*, 1983; Douglas & Shinkai, 1985).

Molecular cloning of glyoxalase I. The genes for glyoxalase I in *S. cerevisiae* and in *Pseudomonas putida* have been cloned and their phenotypic characteristics determined.

The gene for yeast glyoxalase I was cloned onto the YEp13 plasmid. The hybrid plasmid, pY617, tranformed haploid *S. cerevisiae* cells. Transformed cells had 4–5-fold increased glyoxalase I activity, resistance to methylglyoxal toxicity and increased cell size relative to control transformed cells with YEp13 (Murata *et al.*, 1988).

The gene for glyoxalase I in *P. putida* was cloned onto the vector plasmid pBR322. The resulting plasmid, pGI423, transformed *E. coli* cells. Transformed cells had a 150-fold increase in glyoxalase I activity and resistance to methylglyoxal toxicity (Rhee *et al.*, 1987).

The nucleotide sequence of the glyoxalase I gene from *P. putida* has been determined and the amino acid sequence deduced. The molecular mass of the enzyme from the nucleotide sequence is 18442 (20000 estimated from the purified enzyme). The *N*-terminal amino acid sequence determined with the purified enzyme is Ser-Leu-Asp-Asn, whereas the nucleotide sequence indicates Met-Ser-Leu-Asp-Asn. This suggests some post-translational processing of the *N*-terminal methionine residue (Rhee *et al.*, 1988).

Glyoxalase II

Distribution and molecular characteristics. Enzymic activity of glyoxalase II is found throughout biological life. It is found in cell cytosols and in mitochondria but its presence in other organelles is less well-established (Jerzykowski *et al.*, 1978; Talesa *et al.*, 1988; Uotila, 1989).

The molecular mass of the enzyme is in the range 18–29 kDa. The enzyme is a monomer. Glyoxalase II is a basic protein with pI values of 7.4–9.7 depending on the source. In rat liver, 10–15 % of total glyoxalase II activity is in the mitochondria. The cytosolic enzyme consists of two forms of molecular mass 17 and 29 kDa, and pI values between 7.4 and 8.1 (Principato *et al.*, 1987*b*; Talesa *et al.*, 1988) (Table 2).

Substrate specificity. Glyoxalase II can catalyse the hydrolysis of a wide range of *S*-D-hydroxyacylglutathione derivatives. With *S*-lactoyl [$CH_3CH(OH)CO$-], *S*-glycolyl ($HOCH_2CO$-), *S*-mandelyl [PhCH(OH)CO-], *S*-glyceroyl [$HOCH_2CH(OH)CO$-] and *S*-acetoacetyl (CH_3COCH_2CO-) glutathiones, the relative kinetic activity with glyoxalase II is maintained during purification. Pure glyoxalase II is inactive with thioesters of CoA and thioglycollate, indicating a high specificity for the glutathione moiety (Uotila, 1973).

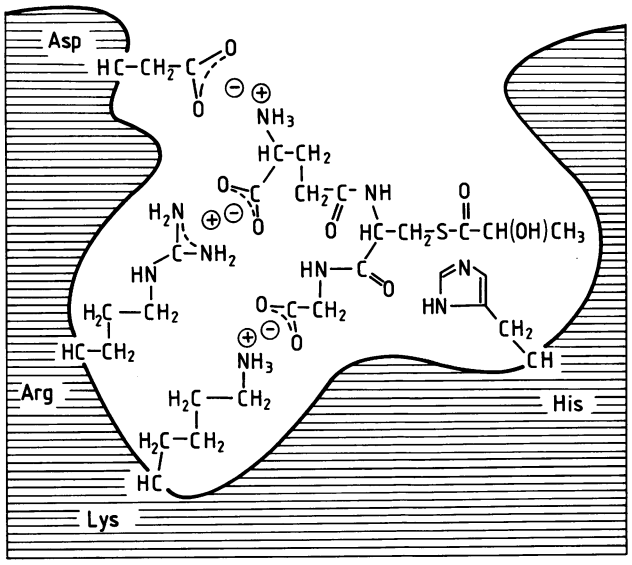
Rate of the glyoxalase II-catalysed reaction. Glyoxalase II catalyses the hydrolysis of *S*-2-hydroxyacylglutathione derivatives to reduced glutathione and the corresponding aldonic acids. The rate of reaction follows Michaelis–Menten kinetics in the substrate range 3–300 μM. *S*-D-Lactoylglutathione is considered to be the physiological substrate and has a K_m value in the range 180–440 μM with glyoxalase II from various sources, except the K_m value for yeast (*Saccharomyces cerevisiae*) glyoxalase II is extraordinarily low, 7 μM for *S*-D-lactoylglutathione (Murata *et al.*, 1986*a*). The value of $k_{cat.}$ is $(16–17) \times 10^3$ min^{-1}, and the value of $k_{cat.}/K_m$ is, in large part, diffusion-limited in some cases. This has suggested that glyoxalase II is a very efficient catalyst for hydrolysis of *S*-D-lactoylglutathione (Guha *et al.*, 1988).

Structure of the active site and catalytic mechanism. Chemical modification studies of glyoxalase II have indicated the presence of arginine, histidine and lysine residues in the active site of glyoxalase II (Ball & Vander Jagt, 1981; Principato *et al.*, 1985; Uotila, 1973). The mechanism proposed for the glyoxalase II-catalysed reaction involves an active site histidine residue.

Table 2. Molecular properties of glyoxalase II

References: Ball & Vander Jagt (1979), Oray & Norton (1980), Murata *et al.* (1986*a*), Principato *et al.* (1984, 1985, 1987*a*,*b*), Talesa *et al.* (1988, 1989), Uotila (1973, 1979).

Source	Molecular mass (kDa)	Isoelectric point (pH)	Kinetic constants for S-D-lactoylglutathione	
			K_m (μM)	$k_{cat.}$ (min^{-1})
Human liver	22.9	8.35	190	–
Human brain	24	8.50	304	–
Human erythrocyte	21	7.7–8.4	–	–
Calf brain	23	7.63	325	16×10^3
Rat erythrocyte	21.9	–	180	17×10^3
Rat liver	24	7.4, 8.0	351, 361	–
Rat liver mitochondria	27, 29	6.6, 6.85, 7.0, 7.5, 8.1	440	–
Mouse liver	29.5	8.10	270	–
Rabbit liver	18–23	7.76	330	–
Chicken liver		6.79, 7.60	180, 150	–
Frog liver		8.20, 8.40, 8.70	320, 450, 440	–
Snake liver		9.25	230	–
Fish liver		9.70	210	–
Yeast (*Saccharomyces cerevisiae*)	19	–	7.0	–



Putative lactoylation mechanism

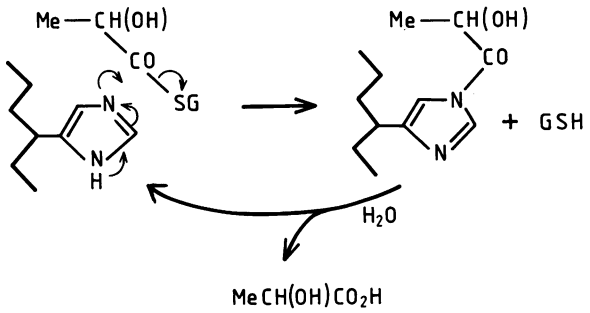


Fig. 5. Putative active site and the catalytic mechanism of glyoxalase II

Active site amino acid residues were deduced from chemical modification studies. A cation recognition site [Asp (shown) or Glu] is inferred from the charge on the substrate.

Histidine is lactoylated by *S*-D-lactoylglutathione, glutathione leaves the active site and the acyl-enzyme intermediate hydrolyses to form D-lactate and the active enzyme (Ball & Vander Jagt, 1981). This is in keeping with the nucleophilicity of the imidazole group and the reactivity of *N*-acylimidazoles towards hydrolysis (Hall *et al.*, 1978; Jencks & Carriuolo, 1959) (Fig. 5). The difference in the kinetics of hydrolysis of *S*-D- and *S*-L-lactoylglutathione (Wieland *et al.*, 1956*b*) suggests some interactions between the α-hydroxyl group of the lactoyl moiety with the enzyme active site.

Genetics and polymorphism. The gene for glyoxalase II in humans is on chromosome 16 (Honey & Shows, 1981). There is usually only one phenotype expressed, although a rare second form (frequency 0.016) was found in a Micronesian population (Board, 1980). Glyoxalase II polymorphism deserves further investigation, however, since one of only two published surveys (Charlesworth, 1972) used inappropriate staining agents.

Inhibition of glyoxalase II. *S*-Carbobenzoxyglutathione derivatives are competitive inhibitors of glyoxalase II (Principato *et al.*, 1989; Al-Timari & Douglas, 1986). Rat liver glyoxalase II is inhibited by *o*-, *m*- and *p*-nitrocarbobenzoxyglutathiones, where the K_i values are 15, 9 and 6.5 μM respectively (Bush & Norton, 1985).

S-D-LACTOYLGLUTATHIONE: AN IMPORTANT INTERMEDIATE IN CHARACTERIZING THE FUNCTION OF THE GLYOXALASE SYSTEM

S-D-Lactoylglutathione is formed by the catalytic action of glyoxalase I and is hydrolysed by the catalytic action of glyoxalase II in the cytosol of cells and cellular organelles. γ-Glutamyltranspeptidase (EC 2.3.2.2) also metabolizes *S*-D-lactoylglutathione to *S*-D-lactoylcysteinylglycine which rearranges to *N*-lactoylcysteinylglycine (Tate, 1975). It seems plausible that the function of *S*-D-lactoylglutathione is associated with the reactive thioester group. The action of γ-glutamyltranspeptidase, present on the external surface of cell plasma membranes and in blood plasma, therefore confines the locus of activity of *S*-D-lactoylglutathione to the interior of cells.

S-D-Lactoylglutathione induces some extraordinary and unexpected biological responses: it potentiates the GTP-promoted assembly of microtubules from porcine brain microtubular protein over the physiological concentration range (Hooper, 1988) and influences neutrophil movement and stimulus-induced secretion of granules (Thornalley, 1989). Moreover, addition of *S*-D-lactoylglutathione to HL60 leukaemia cells in culture induces growth arrest and toxicity (Thornalley & Tisdale, 1988). We have also found increased *S*-D-lactoylglutathione formation during hyperglycaemia associated with diabetes mellitus and the development of clinical complications was correlated to a propensity to maintain high cellular levels of *S*-D-lactoylglutathione (Thornalley *et al.*, 1989). Kimura and co-workers have recently found functional regulation of *S*-D-lactoylglutathione in yeast cells (Murata *et al.*, 1989; Inoue *et al.*, 1989). Research is now in progress studying the effects of *S*-D-lactoylglutathione and analogues, and pharmacological agents which influence *S*-D-lactoylglutathione concentration to define the biological fate and function of *S*-D-lactoylglutathione and to put this to therapeutic benefit.

THE ENERGETIC ARGUMENT FOR THE EXISTENCE OF A GLYOXALASE SYSTEM IN BIOLOGICAL LIFE AND THE REACTIVITY OF *S*-D-LACTOYLGLUTATHIONE

The need for the glyoxalase system in biological metabolism probably stems from the chemistry of dihydroxyacetone phosphate and the presence of reduced glutathione in cell systems. The chemical and biochemical properties of dihydroxyacetone phosphate provide for non-enzymic and enzymic formation of methylglyoxal, and the presence of reduced glutathione affords the formation of hemithioacetal (Fig. 2). Since the equilibrium for hemithioacetal formation lies strongly over to the product, reduced glutathione would be increasingly withdrawn from other cellular functions for hemithioacetal formation if there was no glyoxalase system. The hemithioacetal may be excreted but at the loss of cellular glutathione. Hence, evolutionary pressures have preserved the glyoxalase system. The necessity for a cellular glyoxalase system is implied by the energetics of methylglyoxal and hemithioacetal formation (Creighton *et al.*, 1988).

S-D-Lactoylglutathione is now the focus of future glyoxalase research. Little is known of its reactivity with proteins, but from the chemical model studies of the thioesters with imidazole (Jencks & Carriuolo, 1959; Hall *et al.*, 1978), it is expected that reactive histidyl residues in proteins proximate to a suitable recognition site for the glutathione moiety will describe and define the locus of *S*-D-lactoylglutathione biological reactivity.

Szent-Gyorgyi (1977) proposed that methylglyoxal may change the electronic nature of proteins by binding and modifying susceptible amino acid residues, and thereby influencing cellular

function. It would be a fitting tribute to the great man if proteins are modified by the glyoxalase system but by *S*-D-lactoylglutathione rather than methylglyoxal.

I thank the Medical Research Council, Cancer Research Campaign, Leukaemia Research Fund, Wellcome Trust, Science and Engineering Research Council and ICI Pharmaceuticals for financial support of my research.

REFERENCES

Alexander, N. M. & Boyer, J. L. (1971) Cancer Res. **31**, 1875–1878
Al-Timari, A. & Douglas, K. T. (1986) Biochim. Biophys. Acta **870**, 219–225
Apple, M. A. & Greenberg, D. M. (1967) Cancer Chemother. Rep. **51**, 455–464
Aronsson, A.-C. & Mannervik, B. (1977) Biochem. J. **165**, 503–509
Aronsson, A.-C., Sellin, S., Tibbelin, G. & Mannervik, B. (1981) Biochem. J. **197**, 67–75
Bagga, S., Das, R. & Sopory, S. K. (1987) J. Plant Physiol. **129**, 149–153
Bakker, E., Pearson, P., Meera Kahn, P., Schrender, G. & Madam, K. (1979) Clin. Genet. **15**, 198–202
Ball, J. C. & Vander Jagt, D. L. (1979) Anal. Biochem. **98**, 472–477
Ball, J. C. & Vander Jagt, D. L. (1981) Biochemistry **20**, 899–905
Baskaran, S. & Balasubramanian, K. A. (1987) Biochim. Biophys. Acta **913**, 377–385
Behrens, O. K. (1941) J. Biol. Chem. **141**, 503–508
Bender, K. & Greschik, K. H. (1976) Cytogenet. Cell. Genet. **16**, 93–96
Board, P. G. (1980) Am. J. Human Genet. **32**, 690–694
Brambilla, G., Sciaba, L., Faggin, P., Finollo, R., Bassi, A. M., Ferro, M. & Marinari, U. M. (1985) Carcinogenesis **6**, 683–686
Bush, P. E. & Norton, S. J. (1985) J. Med. Chem. **28**, 828–830
Cambion-de Mouzon, A., Ohayon, E., Hauptmann, G., Seven, A., Abbal, M., Sommer, E., Vergnes, H. & Ducos, J. (1982) Tissue Antigens **19**, 366–379
Carnegie, P. R. (1963) Biochem. J. **89**, 471–478
Carrington, S. J. & Douglas, K. T. (1985) IRCS Med. Sci. **13**, 883–884
Carrington, S. J. & Douglas, K. T. (1986) IRCS Med. Sci. **14**, 763–768
Carrington, S. J. & Douglas, K. T. (1988) J. Chem. Soc. Perkin Trans. II, 2071–2076
Casazza, J. P., Felver, M. E. & Veech, R. L. (1984) J. Biol. Chem. **259**, 231–236
Charlesworth, D. (1972) Am. Hum. Genet. **35**, 477–484
Cheung, S. T. & Fonda, M. L. (1979) Biochem. Biophys. Res. Commun. **90**, 940–947
Conroy, P. J. (1979) Ciba Found. Symp. **67**, 271–298
Cooper, R. A. (1984) Annu. Rev. Microbiol. **38**, 49–68
Creighton, D. J., Migliorini, M., Pourmotabbed, T. & Guha, M. K. (1988) Biochemistry **27**, 7376–7384
Dakin, H. D. & Dudley, M. W. (1913) J. Biol. Chem. **14**, 155–157
Darling, T. N. & Blum, J. (1988) Mol. Biochem. Parasitol. **28**, 121–128
Dixit, A., Garg, L. C., Sutrave, P. & Rao, A. R. (1983) Biochem. Int. **7**, 207–213
Douglas, K. T. (1987) Adv. Enzymol. **59**, 103–167
Douglas, K. T. & Shinkai, S. (1985) Angew. Chem. Int. Engl. Ed. **24**, 31–44
Douglas, K. T., Seddon, A. P. & Nakagawa, Y. (1986) Int. J. Biochem. **18**, 549–555
Dudani, A. K., Srivastava, L. K. & Prasad, R. (1984) Biochim. Biophys. Res. Commun. **119**, 962–967
Egyud, L. G. & Szent-Gyorgyi, A. (1966*a*) Proc. Natl. Acad. Sci. U.S.A. **55**, 388–393
Egyud, L. G. & Szent-Gyorgyi, A. (1966*b*) Proc. Natl. Acad. Sci. U.S.A. **56**, 203–207
Egyud, L. G. & Szent-Gyorgyi, A. (1968) Science **160**, 1140
Ekwall, K. & Mannervik, B. (1970) Arch. Biochem. Biophys. **137**, 128–132
Faggin, P., Bassi, A. M., Finollo, R. & Brambilla, G. (1985) Mutat. Res. **144**, 189–191
Fodor, G., Mijumdar, R. & Szent-Gyorgyi, A. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 4317–4319
Fraval, H. N. & McBrien, D. C. H. (1980) J. Gen. Microbiol. **117**, 127–134
French, F. A. & Freelander, B. L. (1958) Cancer Res. **18**, 172–175
Fung, K. & Grosjean, D. (1981) Anal. Chem. **53**, 168–171
Gillespie, E. (1979) Nature (London) **277**, 135–137
Gillespie, E. (1981) Biochem. Biophys. Res. Commun. **98**, 463–470
Guha, M. K., Vander Jagt, D. L. & Creighton, D. J. (1988) Biochemistry **27**, 8818–8822
Hall, S. S., Doweyko, A. M. & Jordan, F. (1978) J. Am. Chem. Soc. **100**, 5934–5939
Han, L.-P. B., Davison, L. M. & Vander Jagt, D. L. (1977) Biochim. Biophys. Acta **445**, 486–499
Honey, N. K. & Shows, T. B. (1981) Hum. Genet. **58**, 358–361
Hooper, N. I. (1988) Methylglyoxal, Glyoxalases and Control of Cell Proliferation, Ph.D. Thesis, Aston University, Birmingham
Hooper, N. I., Tisdale, M. J. & Thornalley, P. J. (1987) Leuk. Res. **11**, 1141–1148
Hooper, N. I., Tisdale, M. J. & Thornalley, P. J. (1988*a*) Biochim. Biophys. Acta **966**, 362–369
Hooper, N. I. Tisdale, M. J. & Thornalley, P. J. (1988b) Cell. Mol. Biol. **34**, 399–405
Hopkins, F. G. & Morgan, E. J. (1945) Biochem. J. **39**, 320–324
Hopper, D. J. & Cooper, R. A. (1972) Biochem. J. **128**, 321–329
Inoue, Y., Bong-Young, C., Murata, K. & Kimura, A. (1989) Biochem. Biophys. Res. Commun. **165**, 1091–1095
Jencks, W. P. & Carriuolo, J. (1959) J. Biol. Chem. **234**, 1272–1279
Jerzykowski, T., Matuszewski, W., Otrzonsek, N. & Winter, R. (1970) Neoplasma **17**, 25–35
Jerzykowski, T., Matuszewski, W., Tarnawski, R., Winter, R., Herman, Z. S. & Sokola, A. (1975*a*) Arch. Immunol. Therap. Exp. **23**, 549–560
Jerzykowski, T., Winter, R., Matuszewski, W. Szczurek, Z. (1975*b*) Experientia **15**, 32–33
Jerzykowski, T., Winter, R., Matuszewski, W. & Piskorska, D. (1978) Int. J. Biochem. **9**, 853–860
Jordan, F., Cohen, J. F., Wang, C.-T., Wilmott, J. M., Hall, S. S. & Foxall, D. L. (1983) Drug Metab. Rev. **14**, 723–740
Kirk, R. L., Theophilus, J., Whitehouse, S., Court, J. & Zimmet, P. (1979) Diabetes **28**, 949–951
Kirk, R. L., Ranford, P. R., Serjeantson, S. W., Thompson, A. R., Munirathnam-Chetty, S. M., John, L., Mohan, V., Ramachandran, A., Sneholatha, C. & Viswanathan, M. (1985) Diabetes Res. Clin. Pract. **1**, 41–47
Klebanoff, S. J. & Clark, R. A. (1978) The Neutrophil: Function and Clinical Disorders, Elsevier, Amsterdam
Kompf, J., Bissbort, S., Gussmann, S. & Ritter, H. (1975*a*) Humangenetik **27**, 141–143
Kompf, J., Bissbort, S. & Ritter, H. (1975*b*) Humangenetik **28**, 249–251
Kompf, J., Bissbort, S. & Schunter, F. (1976) Hum. Genet. **32**, 197–198
Kozarich, J. W. & Deegan, L. (1979) J. Biol. Chem. **254**, 9345–9348
Krymkiewicz, N. (1973) FEBS Lett. **29**, 51–54
Larsen, K., Aronsson, A.-C., Marmstal, E. & Mannervik, B. (1985) Comp. Biochem. Physiol. **82B**, 625–638
Leach, R., DeMars, R., Hasstedt, S. & White, R. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 3909–3913
Leonici, G., Maresca, M. & Buzzi, E. (1989) Cell. Biochem. Funct. **7**, 65–70
Lozano, A. M. & Mezl, V. A. (1984) Biosci. Rep. **4**, 783–788
Mannervik, B. (1980) Enzymatic Basis of Detoxication (Jacoby, W., ed.) vol. 2, pp. 263–293, Academic Press, New York
Mannervik, B., Marmstal, E., Ekwall, K. & Gorna-Hall, B. (1975) Eur. J. Biochem. **33**, 327–333
Marinari, U. M., Ferro, M., Sciaba, L., Finollo, R., Bassi, A. M. & Brambilla, G. (1984) Cell. Biochem. Funct. **2**, 243–248
Marmstal, E. & Mannervik B. (1978) FEBS Lett. **85**, 275–278
Marmstal, E. & Mannervik, B. (1979) Biochim. Biophys. Acta **566**, 362–370
Marmstal, E. & Mannervik, B. (1981) FEBS Lett. **131**, 301–304
Marmstal, E., Aronsson, A.-C. & Mannervik, B. (1979) Biochem. J. **183**, 23–30
McCann, V. J., Davis, R. E., Welbourn, T. A., Constable, J. & Beale, D. G. (1981) Aust. N.Z. J. Med. **11**, 380–382
McLellan, A. C. & Thornalley, P. J. (1989) Mech. Ageing Dev. **48**, 63–71
Miglietta, A. & Gabriel, L. (1986) Res. Commun. Chem. Pathol. Pharmacol. **51**, 245–260
Murata, K. & Kumura, A. (1986) Nucleic Acid Enzymes **31**, 1010–1021
Murata, K. & Kimura, A. (1987) Sulphur Aminoacids **10**, 187–194
Murata, K., Inoue, Y., Watanabe, K., Fukuda, Y., Saikusa, T. & Kimura, A. (1986*a*) Agric. Biol. Chem. **50**, 135–142
Murata, K., Saikusa, T., Watanabe, K., Fukuda, Y., Shimosaka, M., Inoue, Y. & Kimura, A. (1986*b*) Eur. J. Biochem. **57**, 297–301

Murata, K., Saikusa, T., Inoue, Y. & Kimura, A. (1988) J. Ferment. Technol. **66**, 495–500
Murata, K., Sato, N., Inoue, Y. & Kimura, A. (1989) Agric. Biol. Chem. **53**, 1988–2000
Neuberg, C. (1913) Biochem. Z. **49**, 502–506
Ohmori, S., Mori, M., Kawase, M. & Tsuboi, S. (1987*a*) J. Chromatogr. **414**, 149–155
Ohmori, S., Kawase, M., Mori, M. & Hirota, T. (1987*b*) J. Chromatogr. **415**, 221–229
Ohmori, S., Mori, M., Shirahi, K. & Kawase, M. (1989) in Enzymology and Molecular Biology of Carbonyl Metabolism **2**, pp. 397–412, Liss, New York
Oray, B. & Norton, S. J. (1980) Biochim. Biophys. Acta **611**, 168–173
Patthy, L. (1978) Eur. J. Biochem. **88**, 181–196
Penninckx, M. J., Jaspers, C. J. & Legrain, M. J. (1983) J. Biol. Chem. **258**, 6030–6038
Principato, G. B., Bodo, M., Biagioni, M. G., Rosi, G. & Liotti, F. S. (1982) Acta Embryol. Morphol. Exp. New Ser. **3**, 173–179
Principato, G. B., Locci, P., Rosi, G., Talesa, V. & Giovannini, E. (1983) Biochem. Int. **6**, 248–255
Principato, G. B., Rosi, G., Talesa, V., Bocchini, V. & Giovannini, E. (1984) Biochem. Int. **9**, 351–359
Principato, G. B., Rosi, G., Talesa, V. & Giovannini, E. (1985) IRCS Med. Sci. **13**, 952–953
Principato, G. B., Rosi, G., Talesa, V. & Giovannini, E. (1987*a*) Enzyme **37**, 164–168
Principato, G. B., Rosi, G., Talesa, V., Giovannini, E. & Uotila, L. (1987*b*) Biochim. Biophys. Acta **911**, 349–355
Principato, G. B., Talesa, V., Norton, S. J. & Giovannini, E. (1989) Enzyme **41**, 175–180
Racker, E. (1951) J. Biol. Chem. **190**, 685–696
Racker, E. (1954) Glutathione (Colowick, S. *et al.*, eds.), pp. 165–183, Academic Press, New York
Ramaswamy, O., Pal, S., Guha-Mukerjee, S. & Sopory, S. K. (1983*a*) Biochem. Int. **7**, 307–318
Ramaswamy, O., Pal, S., Sopory, S. K. & Mukerjee, S. (1983*b*) Basic Life Sci. **22**, 43–46
Ramaswamy, O., Pal, S., Guha-Mukerjee, S. & Sopory, S. K. (1984) Plant Cell Rep. **3**, 121–124
Ray, S. & Ray, M. (1983) J. Biol. Chem. **258**, 3461–3462
Ray, M. & Ray, S. (1987) J. Biol. Chem. **262**, 5974–5977
Reeves, H. C. & Ajl, S. J. (1965) J. Biol. Chem. **240**, 568–573
Reichard, G. A., Skutches, C. L., Hoeldtke, R. D. & Owen, O. E. (1986) Diabetes **35**, 668–674
Rhee, H.-I., Murata, K. & Kimura, A. (1986) Biochem. Biophys. Res. Commun. **141**, 993–999
Rhee, H.-I., Murata, K. & Kimura, A. (1987) Biochem. Biophys. Res. Commun. **147**, 831–838
Rhee, H.-I., Murata, K. & Kimura, A. (1988) Agric. Biol. Chem. **52**, 2243–2246
Richard, J. P. (1985) Biochemistry **24**, 949–953
Riddle, V. & Lorenz, F. W. (1969) J. Biol. Chem. **243**, 2718–2724
Sato, J., Wang, J.-M. & Van Eys, J. (1980) J. Biol. Chem. **255**, 2046–2050
Schauenstein, E., Esterbauer, H. & Zollner, H. (1977) in Aldehydes in Biological Systems, pp. 112–157, Pion, London
Schasteen, C. S. & Reed, D. J. (1983) Biochim. Biophys. Acta **742**, 419–425
Sellin, S., Aronsson, A.-C., Eriksson, L. E. G., Larsen, K., Tibbelin, G. & Mannervik, B. (1983) Functions of Glutathione, pp. 187–197, Raven Press, New York
Sethi, U., Basu, A. & Guha-Mukerjee, S. (1988) Plant Sci. **56**, 167–175
Shapiro, R., Cohen, B. I., Shiuey, S.-J. & Maurer, H. (1969) Biochemistry **8**, 238–245
Stohlmacher, P. & Haferland, W. (1980) Z. Rechtsmed. **85**, 165–168
Sutrave, P. & Rao, A. R. (1982) Adv. Life Sci. **1**, 141–145
Szent-Gyorgyi, A. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 2844–2847
Takahashi, K. (1977*a*) J. Biochem. (Tokyo) **81**, 395–402
Takahashi, K. (1977*b*) J. Biochem. (Tokyo) **81**, 403–414
Talesa, V., Uotila, L., Koivusalo, M., Principato, G. B., Giovannini, E. & Rosi, G. (1988) Biochim. Biophys. Acta **955**, 103–110
Talesa, V., Uotila, L., Koivusalo, M., Principato, G. B., Giovinnini, E. & Rosi, G. (1989) Biochim. Biophys. Acta **993**, 7–11
Tate, S. S. (1975) FEBS Lett. **54**, 319–322
Taylor, R. & Agius, L. (1988) Biochem. J. **250**, 625–640
Thornalley, P. J. (1985) Environm. Health Persp. **64**, 297–307
Thornalley, P. J. (1986) in Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine (Rotilio, G., ed.), pp. 481–483, Elsevier, Amsterdam
Thornalley, P. J. (1988) Biochem. J. **254**, 751–755
Thornalley, P. J. (1989) The Glyoxalase System: Towards Functional Characterisation and a Role in Disease Processes, in Handbook on Glutathione, CRC Press, Boca Raton, FL, in the press
Thornalley, P. J. & Atkins, T. W. (1989) Med. Sci. **17**, 777–778
Thornalley, P. J. & Bellavite, P. (1987) Biochim. Biophys. Acta **931**, 120–129
Thornalley, P. J. & Tisdale, M. J. (1988) Leuk. Res. **12**, 897–904
Thornalley, P. J., Wolf, S. P., Crabbe, M. J. C. & Stern, A. (1984) Biochim. Biophys. Acta **797**, 276–287
Thornalley, P. J., Della Bianca, V., Bellavite, P. & Rossi, F. (1987) Biochem. Biophys. Res. Commun. **145**, 769–774
Thornalley, P. J., Hooper, N. I., Florkowski, C., Jones, A. P., Lunec, J. & Barnett, A. H. (1989) Diabetes Res. Clin. Pract. **7**, 115–120
Tokunaga, K., Omoto, K., Juji, T. & Itoh, T. (1982) Human Genet. **62**, 86–88
Tsai, P.-K. & Gracy, R. W. (1976) J. Biol. Chem. **251**, 364–367
Uotila, L. (1973) Biochemistry **12**, 3944–3951
Uotila, L. (1979) Biochim. Biophys. Acta **580**, 277–288
Uotila, L. (1989) in Glutathione: Chemical, Biochemical and Medical Aspects, Part A (Coenzymes and Cofactors) (Dolphin, D., Poulson, R. & Avramovic O., eds.), vol. 2, pp. 767–804, Wiley-Interscience, New York
Vander Jagt, D. L., Daub, F., Krohn, J. A. & Han, L.-P. B. (1975) Biochemistry **14**, 3669–3675
Vogt, M. (1929) Biochem. Z. **211**, 17–39
Weaver, R. H. & Lardy, H. A. (1961) J. Biol. Chem. **236**, 313–317
Wieczorek, E. & Dobosz, T. (1979) Gin. Pol. **50**, 23–29
Wieland, T., Pfleiderer, G. & Lau, H. H. (1956*a*) Biochem. Z. **327**, 393–406
Wieland, T., Sandmann, B. & Pfleiderer, G. (1956*b*) Biochem. Z. **328**, 239–244