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Enzymic imbalance in serine metabolism in rat hepatomas

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The activity of 3-phosphoglycerate dehydrogenase was high in tissues of high cell-renewal capacity, and was increased in neonatal and regenerating liver and, more markedly, in hepatomas. Serine hydroxymethyltransferase activity was present in hepatomas, whereas other enzymes of serine utilization (serine dehydratase and serine aminotransferase) were absent. This enzymic imbalance couples serine biosynthesis preferentially to nucleotide precursor formation in cancer cells.

INTRODUCTION

Serine is a key intermediate in metabolism, providing nitrogen and/or carbon for a variety of products such as glucose, protein, nucleotides and phospholipids. It is generally considered that the biosynthesis *de novo* of this nutritionally dispensable amino acid occurs from glucose via phosphorylated intermediates, with an analogous non-phosphorylated route serving as one of a number of possible alternative pathways of serine utilization [1, 2]. The enzyme 3-phosphoglycerate dehydrogenase (EC 1.1.1.95) catalyses the first step in the biosynthetic pathway. Whereas the bacterial enzyme is feedback-inhibited directly by serine, in animals controls by this reaction step apparently relies on its equilibrium position, which would favour 3-phosphoglycerate resynthesis when later pathway intermediates accumulate [1]. The non-competitive inhibition of phosphoserine phosphatase by serine in animals allows for a feedback-control system via the accumulation of pathway intermediates. In addition, the concentration of 3-phosphoglycerate dehydrogenase in rat and rabbit liver has been shown to be very sensitive to dietary and hormonal influences [1].

In previous work we have provided evidence that serine may be preferentially diverted towards nucleotide biosynthesis, via serine hydroxymethyltransferase, in proliferating, and particularly in neoplastic, tissues [1–4]. It was suggested that the demands for serine utilization for nucleotide biosynthesis in proliferating tissues might be metabolically coupled to an increased capacity for serine synthesis *de novo*. The present study examines the activity of 3-phosphoglycerate dehydrogenase in normal and proliferating rat tissues, including slowly and rapidly growing transplantable hepatomas, the results of which support the above hypothesis. The measurement of enzyme activities of serine utilization in this study supports previous conclusions [1, 2] that the cancer cell has undergone a reprogramming of gene expression, leading to an integrated imbalance in serine metabolism which confers selective growth advantages to these neoplastic cells.

MATERIALS AND METHODS

Materials

Biochemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and ancillary enzymes were from Boehringer Corp., Indianapolis, IN, U.S.A. Other chemicals and radiochemicals were as detailed previously [4].

Animals

Male Wistar rats were used for the tissue-distribution and differentiating-liver studies. Male ACI/N inbred rats weighing 200 g were used for hepatic-regeneration experiments in which partial hepatectomy was carried out under light diethyl ether anaesthesia by a standard procedure [5]; sham-operated animals served as controls. The same strain of rats carried the fast-growing hepatoma 3924A, whereas the slow-growing hepatoma 20 was carried in male Buffalo rats. The growth rates of the hepatoma lines were measured by the time from subcutaneous transplantation until the tumours reached a diameter of 1.5 cm, and were 66 weeks for hepatoma 20 and 4.3 weeks for hepatoma 3924A. Livers of normal male rats of the appropriate strain were used as the control tissue.

Experimental

Tissue homogenates (10%, w/v) were prepared in unbuffered 0.4 M-sucrose by a 30 s homogenization of the minced tissue with a motor-driven Teflon pestle at 600 rev./min. The cytosol fraction was obtained by centrifugation at 105000 *g* for 30 min at 4 °C and used for the assays of 3-phosphoglycerate dehydrogenase and serine dehydratase. Whole homogenates were used for the assays of serine aminotransferase and serine hydroxymethyltransferase after the addition of 0.5% (final concn.) of Triton X-100 for 30 min at 4 °C. Serine dehydratase, serine aminotransferase and serine hydroxymethyltransferase activities were assayed as described previously [4]. It was confirmed in the present study that all enzyme activities were proportional to the time of

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incubation and to the amount of tissue added, as previously reported [2, 4]. 3-Phosphoglycerate dehydrogenase activity was assayed in a 1.0 ml volume containing 100 μmol of potassium phosphate (pH 7.1), 700 μmol of NaCl, 1 μmol of dithiothreitol, 1 μmol of EDTA, 0.2 μmol of NADH, 0.75 μmol of phosphohydroxypyruvate and tissue cytosol (up to the equivalent of 5 mg of tissue) at 30 °C by monitoring NADH disappearance at 340 nm in a Gilford recording spectrophotometer. The reaction mixture was preincubated without phosphohydroxypyruvate for 4 min to rule out the presence of endogenous activity and, after the addition of substrate, the rate was calculated from the linear progress curve between 6 and 16 min. Enzyme activities are expressed as nmol of product formed/h per mg of cytosolic protein and/or as μmol of product formed/min per g wet wt. of original tissue.

RESULTS

3-Phosphoglycerate dehydrogenase assay

The assay system developed was shown to be optimal for both normal liver cytosol and cytosol from hepatomas 20 and 3924A. For both normal liver and hepatoma cytosols, 3-phosphoglycerate dehydrogenase activity was proportional to amount of tissue added up to 5.0 mg (Fig. 1). Mixing experiments showed that the normal liver and hepatoma cytosol activities were additive. These data show that differences in enzyme activity reflect changes in enzyme concentration and eliminate the possibility that this could be attributable to endogenous activators or inhibitors. In view of the sensitivity of the bacterial enzyme to feedback inhibition by serine, the effect of the amino acid was tested on 3-phosphoglycerate dehydrogenase activity from normal liver and hepatoma 3924A. At 1 mM-L-serine there was negligible effect on activity; at 3 mM, L-serine inhibited enzyme activity by 12% (normal liver) and 15% (hepatoma 3924). Thus there is no evidence for a physiologically relevant feedback inhibition of 3-phosphoglycerate directly by L-serine, a finding in keeping with the enzyme properties from other mammalian sources [6–8].

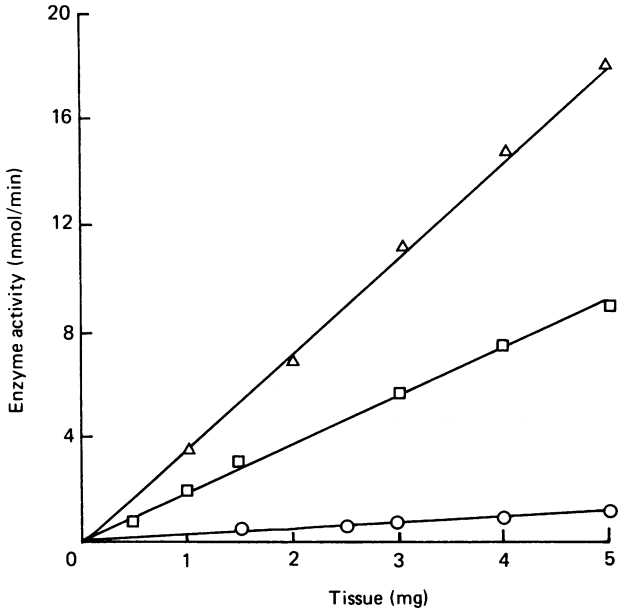

Fig. 1. Proportionality of 3-phosphoglycerate dehydrogenase activity with amount of tissue

3-Phosphoglycerate dehydrogenase activity was assayed with cytosol fractions of ACI/N rat liver (○), hepatoma 20 (□) and hepatoma 3924A (△) as described in the Materials and methods section.

Table 1. Distribution of 3-phosphoglycerate dehydrogenase activity in rat tissues

Activities are expressed on the basis of cytosol protein, wet tissue weight and as a fraction of liver activity (on a wet-weight basis). Means of three or more determinations are shown, ±S.E.M. * Significantly different from liver values ($P < 0.05$ by Student's t test).

Tissue	3-Phosphoglycerate dehydrogenase activity (nmol/h per mg of protein)	(μmol/min per g wet wt.)	Fraction of liver value
Thymus	1333±126	1.16±0.11	12.9*
Spleen	282±28	0.43±0.04	4.8*
Testis	1366±214	1.15±0.18	12.8*
Liver	57±8	0.09±0.01	(1.0)
Lung	36±9	0.05±0.01	0.5*
Renal cortex	1539±27	2.29±0.04	25.4*
Heart	55±9	0.05±0.01	0.6*
Brain	609±83	0.29±0.04	3.2*

3-Phosphoglycerate dehydrogenase activity in rat tissues

The highest activity of 3-phosphoglycerate dehydrogenase was found in the kidney (Table 1), and this agrees with the tissue distribution of serine formation from [^{14}C]glucose [9] and activities of other enzymes of the serine biosynthetic pathway [2, 10]. After the kidney, activity was highest in tissues of high cell-renewal (thymus, testis, spleen) and lowest in tissues of low cell-renewal capacity (lung, heart), relative to that of the liver. The activity was also high in the brain, particularly on a cytosol-protein basis.

3-Phosphoglycerate dehydrogenase activity in differentiating and regenerating liver

3-Phosphoglycerate dehydrogenase activity was elevated 32.8-fold in neonatal rat liver compared with normal adult values (Table 2). The differentiating organ at these ages is actively engaged in cell proliferation; the 5-day-old rats were chosen for the neonatal measurements because by this age the liver is free of haematopoietic cells. The effects of cell proliferation during liver regeneration after partial hepatectomy on 3-phosphoglycerate dehydrogenase activity are also shown in Table 2. The activity was significantly increased 24 h after partial hepatectomy, by 2.6-fold compared with sham-operated control rats. In the same study serine dehydratase activity was not significantly changed compared with control animals (results not shown).

Activities of 3-phosphoglycerate dehydrogenase and enzymes of serine utilization in rat hepatomas

3-Phosphoglycerate dehydrogenase activity was elevated on a protein or wet-weight basis in both the

Table 2. Activity of 3-phosphoglycerate dehydrogenase in differentiating and regenerating rat livers

Activities are expressed on the basis of cytosol protein, wet wt. of liver and as a fraction of control liver activity (on a wet-weight basis). Values in differentiating liver are from Wistar rats and those in regenerating liver are from ACI/N rats. Means of four or more determinations are shown, ±S.E.M. * Significantly different from adult or sham-operated control ($P < 0.01$ by Student's t test).

Liver	3-Phosphoglycerate dehydrogenase activity (nmol/h per mg of protein)	3-Phosphoglycerate dehydrogenase activity (μmol/min per g wet wt.)	Fraction of control value
Adult control	57±8	0.09±0.01	1.0
5-day neonatal	3391±241	2.95±0.21	32.8*
24 h-sham-operated	30±4	0.05±0.007	1.0
24 h-partially hepatectomized	78±15	0.13±0.026	2.6*

slow-growing hepatoma 20 (by 11.9-fold or 8.8-fold) and the fast-growing hepatoma 3924A (75.5-fold or 50.8-fold) compared with normal liver control values (Table 3). Enzyme activity was increased to about a 6-fold greater extent in the faster-growing tumour. This is consistent with the apparent correlation of 3-phosphoglycerate dehydrogenase activity with the growth rate of four other hepatomas in the only other study of this enzyme in neoplastic tissues [11]. Assays were also carried out in hepatomas 20 and 3924A for enzymes of serine utilization previously studied by one of us in other transplantable tumours [2]. Serine dehydratase and serine aminotransferase activities were essentially absent from the hepatomas, whereas serine hydroxymethyltransferase activity was retained at 50% (hepatoma 20) and 19% (hepatoma 3924A) of control liver activity. We have shown that, in a more malignant hepatoma (5123tc) and in a rapidly growing transplantable sarcoma, serine hydroxymethyltransferase activity was increased about 3-fold and 9-fold respectively over values in control liver and muscle tissue (K. Snell & G. Weber, unpublished work).

DISCUSSION

3-Phosphoglycerate dehydrogenase is a key enzyme that catalyses the first committed reaction step in the pathway of serine biosynthesis from glycolytic intermediates. Enzyme activity, in the direction of 3-phosphohydroxypyruvate formation, in rat liver *in vitro* is the lowest of the serine-biosynthetic enzymes, although the situation *in vivo* is less certain because of the unknown extent of phosphoserine phosphatase inhibition by serine [1]. In terms of adaptive regulation of enzyme amount, liver 3-phosphoglycerate dehydrogenase activity is the most sensitive of the pathway enzymes to dietary and hormonal influences [1]. In general, the activity of 3-phosphoglycerate dehydrogenase assayed in the present study in different tissues and in different pathophysiological states correlates positively with increased cellular proliferation. The organ distribution of the enzyme shows

Table 3. Activities of 3-phosphoglycerate dehydrogenase, serine dehydratase, serine aminotransferase and serine hydroxymethyltransferase in rat hepatomas

3-Phosphoglycerate dehydrogenase and serine dehydratase activities were assayed in tissue cytosol fractions; serine aminotransferase and serine hydroxymethyltransferase activities were assayed in whole homogenates (containing 0.5% Triton). Means of three or more determinations are shown, ±S.E.M. All tumour values are significantly different from the appropriate control ($P < 0.01$ by Student's t test).

Tissue	3-Phosphoglycerate dehydrogenase (nmol/h per mg of protein)	3-Phosphoglycerate dehydrogenase (μmol/min per g wet wt.)	Serine dehydratase (μmol/min per g wet wt.)	Serine aminotransferase (μmol/min per g wet wt.)	Serine hydroxymethyltransferase (μmol/min per g wet wt.)
Normal liver control for:					
20	85±4	0.14±0.007	7.69±0.37	0.210±0.013	1.84±0.11
3924A	57±2	0.09±0.004	6.85±1.08	0.208±0.013	1.84±0.08
Hepatoma:					
20	1007±49	1.23±0.06	< 0.02	< 0.01	0.92±0.05
3924A	4305±151	4.57±0.16	< 0.02	< 0.01	0.35±0.004

increased activity in tissues with high cell-renewal capacity. The high activities in brain and kidney are exceptions to this generalization. It may be that in brain and kidney the increased enzyme activity is associated with the demands for serine biosynthesis for the formation of glycine, which is specifically used in those tissues as an inhibitory neurotransmitter and as an ammoniagenic precursor respectively. The activity of 3-phosphoglycerate dehydrogenase is also increased in proliferating liver cells during either the natural differentiation of developing neonatal liver or the restorative hyperplasia which accompanies adult-liver regeneration after partial hepatectomy. But most striking was the increase in enzyme activity in proliferating neoplastic cells in hepatomas 20 and 3924A. Since the activity was increased in both the slow-growing and, to a greater extent, in the rapidly growing hepatomas, the enzyme appears to be linked with both neoplastic transformation and progression.

The magnitude of the increase in 3-phosphoglycerate dehydrogenase in hepatoma 3924A (50-fold, compared with normal adult liver) is much greater than in the other situations of active proliferation. It contrasts particularly with the regenerating liver at 24 h, where the proliferation rate is similar to that of hepatoma 3924A [12], but enzyme activity is only increased 2.6-fold. Furthermore, in the 5-day-old differentiating liver, although activity per g of liver is increased about 30-fold, the increase in activity per cell would only be about 10-fold, given the differences in cellularity between adult and neonatal liver [13]. These factors point to a specificity in the changes of 3-phosphoglycerate dehydrogenase activity in cancer cells. This feature is reinforced by a comparison with enzymes of serine utilization. Only one of the enzymes of serine utilization that was assayed, serine hydroxymethyltransferase, retained activity in the hepatomas; the other enzymes (serine dehydratase and serine aminotransferase) showed no detectable activity. This has been previously shown to be the case in other neoplastic tissues [2]. In contrast, in regenerating liver the increase in 3-phosphoglycerate dehydrogenase activity was not accompanied by any change in serine dehydratase activity. Thus the enzymic shifts in cancer cells are unique in that they involve the co-ordinated increase of the enzymes of serine biosynthesis, i.e. 3-phosphoglycerate dehydrogenase (the present study), phosphoserine aminotransferase [2] and phosphoserine phosphatase [10], with a deletion of two of the enzymes of serine utilization (serine dehydratase and serine aminotransferase), but with a variable retention of serine hydroxymethyltransferase (the present study; [2]). This enzymic imbalance, in comparison with normal tissues, should ensure that any increased serine biosynthesis in cancer cells is preferentially channelled towards its utilization for nucleotide precursor formation in the serine hydroxymethyltransferase reaction.

It has been proposed that an ordered, integrated, imbalance in the metabolism and enzymology of tumour cells is due to a co-ordinated reprogramming of gene expression [12]. In the present study the activities of 3-phosphoglycerate dehydrogenase and the enzymes of serine utilization were measured under optimal conditions, which yielded linear kinetics proportional to the amounts of the enzymes. Given the assumption that enzyme amount is an indicator of the extent of gene expression under steady-state conditions, the present work shows that there is a co-ordinated reprogramming of gene expression which underlies the reorientation of serine metabolism in cancer cells.

In view of the increased glycolytic capacity in various tumour types [4], 3-phosphoglycerate dehydrogenase occupies a key position in diverting glucose-derived carbon towards serine biosynthesis. It has been demonstrated that the increased glycolytic rate in tumours subserves the needs for the provision of precursors of nucleotide biosynthesis, which is a part of the biochemical strategy associated with the commitment to replication of the neoplastic cell [12–14]. Integrated increases in glucose-6-phosphate dehydrogenase, transaldolase and ribose-phosphate pyrophosphokinase ensure that glycolytic intermediates are channelled towards the provision of pentoses and phosphoribosyl pyrophosphate for the increased activities of the purine and pyrimidine nucleotide synthesis pathways in tumours [1, 15]. In the same way the increased activity of 3-phosphoglycerate dehydrogenase in hepatomas could draw on an increased provision of glycolytic intermediates for serine biosynthesis. Metabolism of serine via serine hydroxymethyltransferase, in the absence of alternative pathways of serine utilization in hepatomas, leads to the generation of both glycine and C_1-tetrahydrofolate derivatives, which serve as precursors for purine and pyrimidine biosyntheses. This integrated enzymic imbalance in serine metabolism serves to couple serine biosynthesis metabolically to its utilization for nucleotide formation. Such a metabolic imbalance should confer selective proliferative advantages on cancer cells over their normal somatic counterparts.

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