Biochem. J. (1984) **217**, 615–622
Printed in Great Britain

The oxidation of oxyhaemoglobin by glyceraldehyde and other simple monosaccharides

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(*Received 4 May 1983/Accepted 6 October 1983*)

Glyceraldehyde and other simple monosaccharides oxidize oxyhaemoglobin to methaemoglobin in phosphate buffer at pH 7.4 and 37°C, with the concomitant production of H_2O_2 and an α-oxo aldehyde derivative of the monosaccharide. Simple monosaccharides also reduce methaemoglobin to ferrohaemichromes (non-intact haemoglobin) at pH 7.4 and 37°C. Carbonmonoxyhaemoglobin is unreactive towards oxidation by autoxidizing glyceraldehyde. Free-radical production from autoxidizing monosaccharides with haemoglobins was observed by the e.s.r. technique of spin trapping with the spin trap 5,5-dimethyl-1-pyrroline *N*-oxide. Hydroxyl and 1-hydroxyalkyl radical production observed from monosaccharide autoxidation was quenched in the presence of oxyhaemoglobin and methaemoglobin. The haemoglobins appear to quench the free radicals by reaction with the free radicals and/or the ene-diol precursor of the free radical.

Oxyhaemoglobin is susceptible to oxidation, forming methaemoglobin and H_2O_2. Both reducing and oxidizing agents stimulate this process (Antonini *et al.*, 1965; Castro *et al.*, 1978; reviewed in Wallace *et al.*, 1978). Cogent examples of reductants active in this process are the hydrazine-type drugs, where the hydrazine is oxidized to N_2 along with methaemoglobin formation and O_2 reduction (Goldberg & Stern, 1977*a*; Hill & Thornalley, 1981). Active oxidants are typified by peroxides and oxidized quinones, which are thought to be reduced by oxyhaemoglobin, producing methaemoglobin and reactive oxygen-centred free-radical intermediates (Goldberg & Stern, 1976; Trotta *et al.*, 1981). We now report here the stimulation of oxyhaemoglobin oxidation to methaemoglobin and the reduction of methaemoglobin to ferrohaemichrome by autoxidizing glyceraldehyde and other simple monosaccharides.

Materials and methods

Haemoglobins

Oxyhaemoglobin was prepared from human erythrocytes by published methods (Hennessey *et al.*, 1962). Methaemoglobin and carbonmonoxyhaemoglobin were prepared by published methods (Thornalley *et al.*, 1983*a*).

Monosaccharides

DL-Glyceraldehyde, L-glyceraldehyde, D-glyceraldehyde, dihydroxyacetone, dihydroxyacetone phosphate (lithium salt), glycolaldehyde and methylglyoxal were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Hydroxypyruvaldehyde was synthesized by published methods (Reeves & Ajl, 1965).

Miscellaneous materials

Cu–Zn-superoxide dismutase, prepared from human erythrocytes, was generously given by Dr. J. V. Bannister (University of Oxford, Oxford, U.K.) and had an activity of 3300 units (μmol/min)/mg of protein. Catalase from ox liver was purchased from Calbiochem–Behring Corp. (La Jolla, CA, U.S.A.) and had an activity of 88823 units (μmol/min)/mg of protein. The spin-trapping agent DMPO was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.) and purified by published methods (Bonnett *et al.*, 1959; Beuttner & Oberley, 1978). The metal-ion-chelating ligand DETAPAC was purchased from Sigma.

Abbreviations used: DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DETAPAC, diethylenetriamine-*NNN'N"N"*-penta-acetic acid.

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Visible spectroscopy

Oxyhaemoglobin oxidation by monosaccharides was followed by monitoring the visible-absorption spectrum of the α- and β-bands of oxyhaemoglobin (wavelength range 700–500nm). The decrease of the β-band was monitored at 577nm. Methaemoglobin reduction by monosaccharides was similarly followed by monitoring the decrease in the 630nm band of the visible-absorption spectrum of methaemoglobin. The mean $-\Delta A_{577}/dt$ and $-\Delta A_{630}/dt$ from four determinations are given.

O_2 uptake

O_2 consumption by the reaction of glyceraldehyde with haemoglobin derivatives in aqueous phosphate-buffered solutions (pH7.4) at 37°C was monitored by using a Clark-type oxygen electrode (YSI model 53 oxygen monitor; Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.). Values for O_2 consumption presented are the means for four experiments.

Glyceraldehyde consumption

The consumption of glyceraldehyde during incubation of 50mM-glyceraldehyde with haemoglobin derivatives was monitored by end-point enzymic assay with the use of yeast aldehyde dehydrogenase (purchased from Sigma), as previously described (Rendina & Cleland, 1981).

α-Oxo aldehyde determination

Hydroxypyruvaldehyde was determined in glyceraldehyde autoxidation mixtures as the Girard T adduct ($\varepsilon_{280} = 27\,mM^{-1}\cdot cm^{-1}$) (Cogoli-Greuter & Christen, 1981). This was compared with hydroxypyruvaldehyde production measured as the glutathione adduct ($\varepsilon_{245} = 3.37\,mM^{-1}\cdot cm^{-1}$) formed catalytically in the presence of glyoxalase I/glutathione systems (Bergmeyer *et al.*, 1974).

E.s.r. spectroscopy

E.s.r. spectra were recorded on a Varian E109 X-band dual-cavity e.s.r. spectrometer. Oxyhaemoglobin and methaemoglobin were incubated with 50mM-monosaccharide for 10min at 37°C in the presence of 100mM-DMPO in 100mM-sodium phosphate buffer, pH7.4, and then the e.s.r. spectrum of the incubation mixture was immediately recorded.

Results

Oxidation of oxyhaemoglobin by monosaccharides

Fig. 1 shows the visible-absorption spectra of oxyhaemoglobin, methaemoglobin and carbonmonoxyhaemoglobin when incubated with 50mM-glyceraldehyde. The spectral changes observed from incubation of glyceraldehyde with oxyhaemoglobin (Fig. 1*a*) are characteristic of oxyhaemoglobin oxidation to methaemoglobin. Incubations with methaemoglobin and glyceraldehyde showed spectral changes characteristic of oxyhaemoglobin production from methaemoglobin (Fig. 1*a*), but the increasing 'oxyhaemoglobin' component produced by glyceraldehyde treatment of methaemoglobin could not be deoxygenated to give the typical (see, e.g., Benesch *et al.*, 1973) deoxyhaemoglobin spectrum. This suggests that glyceraldehyde produces a ferrohaemichrome (non-intact haemoglobin). Carbonmonoxyhaemoglobin does not undergo haem redox transitions in the presence of 50mM-glyceraldehyde (Fig. 1*c*).

The mechanism of the reaction of oxyhaemoglobin with glyceraldehyde and other monosaccharides was further investigated by measuring the rates of monosaccharide-induced oxyhaemoglobin oxidation in the presence of various additives (Table 1). Glyceraldehyde (50mM) enhances the rate of oxyhaemoglobin oxidation at 37°C by approx. 20-fold. Addition of DETAPAC (50μM) and of catalase (583 units/ml) suppresses the glyceraldehyde stimulation of oxyhaemoglobin oxidation by 84% and 58% respectively, whereas addition of superoxide dismutase (0.1mg/ml), of dimethyl sulphoxide (a hydroxyl-radical scavenger) (1%, v/v) and of DMPO (spin-trapping agent) (100mM) has no measurable or small effects on the rate of oxyhaemoglobin oxidation stimulated by glyceraldehyde.

Initial-rate measurements show the oxidation of oxyhaemoglobin by glyceraldehyde to be first-order with respect to oxyhaemoglobin and glyceraldehyde (Fig. 2):

$$-d[\text{oxyhaem}]/dt = k_{obs.}[\text{glyceraldehyde}][\text{oxyhaem}]$$

with an observed bimolecular rate constant $k_{obs.} = 8\times10^{-3}\,M^{-1}\cdot s^{-1}$ in 100mM-sodium phosphate buffer, pH7.4, at 37°C. However, the addition of DETAPAC (1mM) decreases the observed rate constant by approx. 50-fold: $k_{obs.}$ (DETAPAC) $= 1.6\times10^{-4}\,M^{-1}\cdot s^{-1}$ in 100mM-sodium phosphate buffer, pH7.4, at 37°C. D-, DL- and L-Glyceraldehyde all give the same rates of oxyhaemoglobin oxidation. Methylglyoxal, glycolaldehyde and hydroxypyruvaldehyde show lower rates of oxidation of oxyhaemoglobin, and dihydroxyacetone shows a slightly faster rate of oxidation of oxyhaemoglobin, than observed with corresponding glyceraldehyde incubations.

Table 1 shows the relative rates of glyceraldehyde-stimulated oxyhaemoglobin oxidation in 100mM-sodium phosphate, -Tris/HCl and -Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/HCl buffers at pH7.4, with and without 1mM-DETAPAC; the order of the rates of oxy-

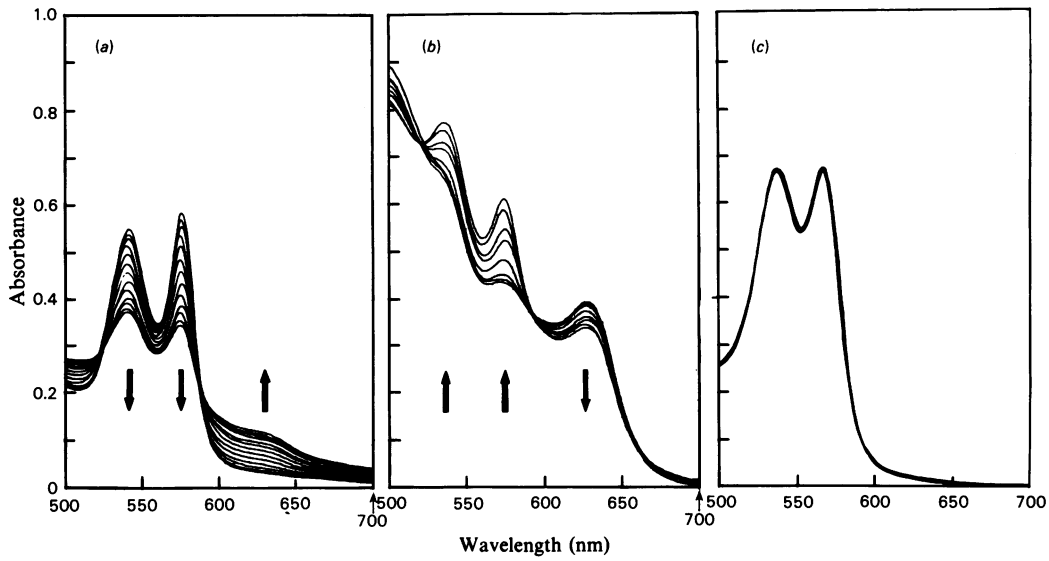


Fig. 1. *Visible-absorption spectra of glyceraldehyde/haemoglobin systems*
(*a*) Oxyhaemoglobin. Reaction mixture contains 50mM-glyceraldehyde and 10μM-oxyhaemoglobin in 100mM-sodium phosphate buffer, pH7.4, at 37°C. Visible-absorption spectra were scanned every 3min from $t = 0$ to $t = 36$min. (*b*) Methaemoglobin. Reaction mixture contains 50mM-glyceraldehyde and 30μM-methaemoglobin in 100mM-sodium phosphate buffer, pH7.4, at 37°C. Visible absorption spectra were scanned at $t = 0$, 5, 10, 20, 30, 40, 50 and 60min. (*c*) Carbonmonoxyhaemoglobin. Reaction mixture contains 50mM-glyceraldehyde and 15μM-carbonmonoxyhaemoglobin in 100mM-sodium phosphate buffer, pH7.4, at 37°C. Visible-absorption spectra were scanned at 0, 30 and 60min. Arrows denote the progression of the absorption bands with time.

Table 1. *Factors affecting the rate of oxidation of oxyhaemoglobin by autoxidizing monosaccharides at 37°C*
All systems contain 10μM-oxyhaemoglobin. Relative initial rates are expressed as the ratio of the initial rate of oxyhaemoglobin oxidation in the system (R^0) to the initial rate of oxyhaemoglobin oxidation by 50mM-DL-glyceraldehyde in 100mM-sodium phosphate buffer, pH7.4, at 37°C (R^0_{GL}). Errors are within $\pm 10\%$ of given values.

Monosaccharide	Buffer system	Other addition	Relative initial rate of oxyhaemoglobin (R^0/R^0_{GL})
None	100mM-Sodium phosphate, pH7.4	None	4
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	None	100
50mM-D-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	None	100
50mM-L-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	None	100
50mM-Dihydroxyacetone	100mM-Sodium phosphate, pH7.4	None	122
50mM-Hydroxypyruvaldehyde	100mM-Sodium phosphate, pH7.4	None	71
50mM-Glycolaldehyde	100mM-Sodium phosphate, pH7.4	None	37
50mM-Methylglyoxal	100mM-Sodium phosphate, pH7.4	None	6
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	Superoxide dismutase (0.1mg/ml)	74
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	Catalase (583units/ml)	42
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	DETAPAC (50μM)	16
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	DMPO (100mM)	68
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	Dimethyl sulphoxide (1%)	99
50mM-DL-Glyceraldehyde	100mM-Sodium phosphate, pH7.4	DETAPAC (1mM)	9
50mM-DL-Glyceraldehyde	100mM-Tris/HCl, pH7.4	None	25
50mM-DL-Glyceraldehyde	100mM-Tris/HCl, pH7.4	DETAPAC (1mM)	4
50mM-DL-Glyceraldehyde	100mM-Hepes/HCl, pH7.4	None	19
50mM-DL-Glyceraldehyde	100mM-Hepes/HCl, pH7.4	DETAPAC (1mM)	3

haemoglobin oxidation is phosphate > Hepes/HCl > Tris/HCl.

The observed rates of glyceraldehyde-mediated reduction of methaemoglobin are given in Table 2. The rate of reduction of methaemoglobin by glyceraldehyde is approx. 4-fold faster in anaerobic than in aerobic conditions. The anaerobic reduction of methaemoglobin by glyceraldehyde is first-order with respect to glyceraldehyde and methaemoglobin:

$$-\mathrm{d[methaem]}/\mathrm{d}t = k_{\mathrm{obs.}}[\text{glyceraldehyde}][\text{oxyhaem}][\text{methaem}]$$

with an observed bimolecular rate constant of $k_{\mathrm{obs.}} = 4 \times 10^{-3}\,\mathrm{M^{-1}\cdot s^{-1}}$ in 100mM-sodium phosphate buffer, pH7.4, at 37°C. Addition of DETAPAC, of superoxide dismutase, of catalase and of superoxide dismutase + catalase together in each case increases the observed rate of methaemoglobin reduction by glyceraldehyde, but all these rates are well below the rate of glyceraldehyde-mediated reduction of methaemoglobin under anaerobiosis.

Effect of haemoglobin derivatives on monosaccharide autoxidation

Glyceraldehyde consumption. Addition of oxyhaemoglobin (10μM) to autoxidizing glyceraldehyde systems increases the rate of loss of glyceraldehyde; addition of carbonmonoxyhaemoglobin (10μM) also stimulates glyceraldehyde consumption, whereas methaemoglobin (10μM) decreases the rate of glyceraldehyde consumption below that

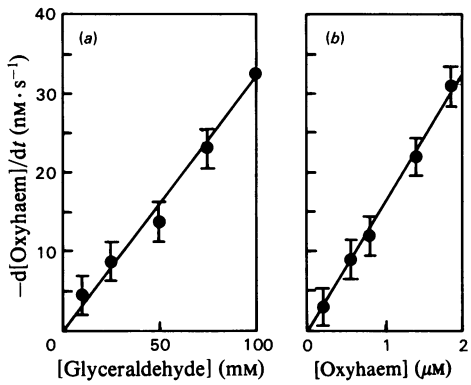


Fig. 2. *Kinetics of the reaction between glyceraldehyde and oxyhaemoglobin*
Rates of decrease in oxyhaemoglobin concentration were monitored as the decrease in the 577nm absorption band in the presence of glyceraldehyde at 37°C.

Table 2. *Factors affecting the rate of reduction of methaemoglobin by glyceraldehyde at 37°C*
All reaction mixtures contained 50mM-DL-glyceraldehyde and 12.5μM-methaemoglobin in 100mM-sodium phosphate buffer, pH7.4, and are aerobic (initial O_2 concentration 178μM) unless otherwise stated. Rates are given on a methaem basis, i.e. for the reaction:

$$\text{glyceraldehyde} + \mathrm{HbFe^{III}} \rightarrow \text{products}$$

Addition to reaction mixture	Rate of methaemoglobin reduction (nM·s⁻¹)
None	2.5
(Anaerobic)	9.3
DETAPAC (1mM)	3.2
Superoxide dismutase (0.1mg/ml)	3.5
Catalase (583units/ml)	4.4
Superoxide dismutase (0.1mg/ml) + catalase (583units/ml)	5.8

Table 3. *Consumption of glyceraldehyde and O_2 with the production of hydroxypyruvaldehyde by glyceraldehyde/haemoglobin systems*
Values are given as means ± S.D. for four determinations.

Reaction mixture	Initial rate at 37°C
Glyceraldehyde consumption:	$-\mathrm{d[Glyceraldehyde]}/\mathrm{d}t$ (μM·s⁻¹)
(i) 50mM-DL-Glyceraldehyde in 100mM-sodium phosphate, pH7.4	0.78±0.10
(ii) As in (i), +10μM-oxyhaemoglobin	1.31±0.10
(iii) As in (i), +10μM-methaemoglobin	0.53±0.10
(iv) As in (i), +10μM-carbonmonoxyhaemoglobin	0.98±0.10
O_2 consumption:	$-\mathrm{d[O_2]}/\mathrm{d}t$ (nM·s⁻¹)
(v) 50mM-DL-Glyceraldehyde + 1mM-DETAPAC in 100mM-sodium phosphate, pH7.4	141±10
(vi) As in (v), +50μM-methaemoglobin	40±5
(vii) As in (v), +50μM-carbonmonoxyhaemoglobin	12±2
(viii) As in (v), +50μM-oxyhaemoglobin	6±1
Hydroxypyruvaldehyde production:	+d[Hydroxypyruvaldehyde]/dt (μM·s⁻¹)
(ix) 50mM-DL-Glyceraldehyde in 100mM-sodium phosphate, pH7.4	0.19±0.03
(x) As in (ix), +10μM-oxyhaemoglobin	0.11±0.02

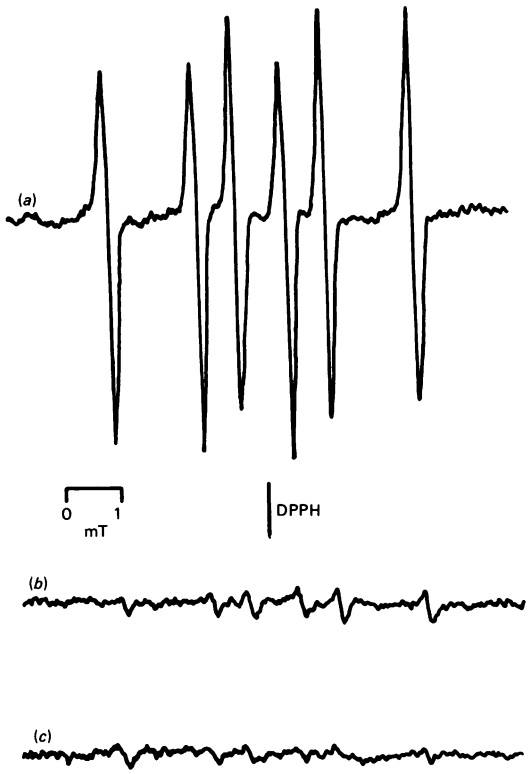


Fig. 3. *Inhibition of free-radical production from autoxidizing glyceraldehyde by haemoglobin derivatives*
Reaction mixtures contained 50mM-glyceraldehyde and 100mM-DMPO in 100mM-sodium phosphate buffer, pH7.4, with: (*a*) no further additions (control); (*b*) 12.5μM-oxyhaemoglobin; (*c*) 12.5μM-methaemoglobin. Reaction mixtures were incubated at 37°C for 10min, and the e.s.r. spectrum of each incubation mixture was immediately recorded. Abbreviation: DPPH, diphenylpicrylhydrazyl.

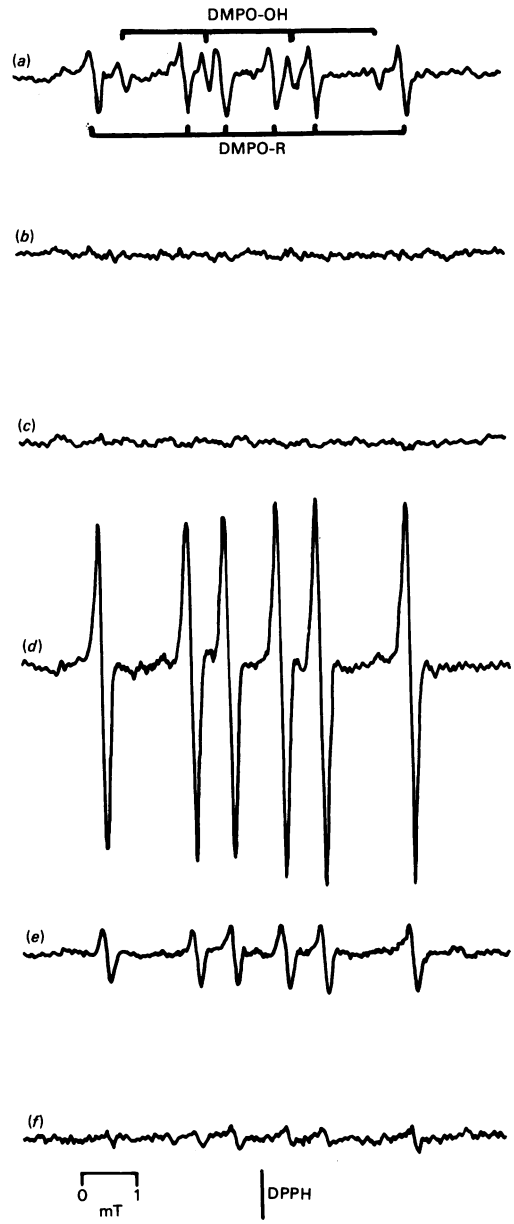


Fig. 4. *Inhibition of free-radical production from autoxidizing dihydroxyacetone and glycolaldehyde by haemoglobin derivatives*
Reaction mixtures contained: (*a*) 50mM-dihydroxyacetone and 100mM-DMPO in 100mM-sodium phosphate buffer, pH7.4; (*b*) as for (*a*), +12.5μM-oxyhaemoglobin; (*c*) as for (*a*) +12.5μM-methaemoglobin; (*d*) 50mM-glycolaldehyde and 100mM-DMPO in 100mM-sodium phosphate buffer, pH7.4; (*e*) as for (*d*) +12.5μM-oxyhaemoglobin; (*f*) as for (*d*), +12.5μM-methaemoglobin. Reaction mixtures were incubated at 37°C for 10min, and the e.s.r. spectrum of each incubation mixture was immediately recorded. Abbreviation: DPPH, diphenylpicrylhydrazyl.

found in the control spontaneous autoxidation (Table 3).

O_2 consumption. Methaemoglobin, carbonmonoxyhaemoglobin and oxyhaemoglobin each decrease the rate of O_2 consumption by autoxidizing glyceraldehyde below that observed in the absence of any haemoglobin derivative (control) (Table 3). The relative rates of O_2 consumption show the order: control > methaemoglobin > carbonmonoxyhaemoglobin > oxyhaemoglobin.

Hydroxypyruvaldehyde production. Hydroxypyruvaldehyde production from glyceraldehyde is decreased by approx. 50% in the presence of 10μM-oxyhaemoglobin (Table 3) over an incubation period of 1h at 37°C.

Free-radical production

At pH7.4 autoxidizing glyceraldehyde in the presence of the spin-trapping agent DMPO gave the e.s.r. spectrum shown in Fig. 3(*a*). This has previously been reported (Thornalley *et al.*, 1983*b*) to be the e.s.r. spectrum of a carbon-centred free-radical-derived spin adduct (DMPO-R). In the presence of 12.5μM-oxyhaemoglobin, however, autoxidizing glyceraldehyde gave a decreased spectral intensity of DMPO-R (approx. 40% decrease; Fig. 3*b*). With 12.5μM-methaemoglobin, the spectral intensity of DMPO-R was further diminished (approx. 45% decrease; Fig. 3*c*). Similar results were obtained for the autoxidation of dihydroxyacetone and glycolaldehyde in the presence of haemoglobins (Fig. 4).

Discussion

Reaction of monosaccharides with haemoglobin

Previous studies (Wolff *et al.*, 1983) have shown that monosaccharides autoxidize through an ene-diol tautomer. The autoxidation of simple monosaccharides was characterized by the loss of monosaccharide, O_2 consumption and α-oxo aldehyde production. Oxyhaemoglobin has previously been shown to be susceptible to oxidation stimulated by ene-diol dihydroxyfumaric acid (Goldberg & Stern, 1977*b*).

In the present paper we have described the oxidation of oxyhaemoglobin by glyceraldehyde and other simple monosaccharides. The oxidation of oxyhaemoglobin by glyceraldehyde appears to be dependent on H_2O_2 (as indicated by the inhibition with addition of catalase) and is catalysed by trace metal ions (as indicated by the effect of metal-ion chelators). Neither the spin trap DMPO nor the hydroxyl-radical scavenger dimethyl sulphoxide inhibited the oxidation of oxyhaemoglobin induced by glyceraldehyde. Glyceraldehyde-induced oxidation of oxyhaemoglobin therefore appears to be promoted by a direct reaction of desoxyhaemoglobin/oxyhaemoglobin with H_2O_2 and the ene-diol of glyceraldehyde.

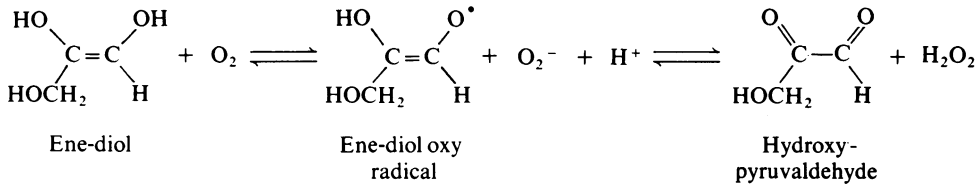
The order of reactivity of the monosaccharides and α-oxoaldehydes towards oxyhaemoglobin oxidation parallels the reactivity found for these compounds towards enolization to an ene-diol and autoxidation (Thornalley *et al.*, 1983*b*): dihydroxyacetone > hydroxypyruvaldehyde = D-glyceraldehyde > DL-glyceraldehyde = L-glyceraldehyde > glycolaldehyde > methylglyoxal.

The effects of buffer ions on the rate of glyceraldehyde-stimulated oxyhaemoglobin oxidation parallel the influence of buffer ions on the rate of monosaccharide autoxidation (Thornalley *et al.*, 1983*b*). This suggests that the intermediates and products of monosaccharide autoxidation induce oxidation of oxyhaemoglobin by glyceraldehyde. Buffer-ion effects on monosaccharide autoxidation were found to be exerted through influences on the rate of monosaccharide enolization; this implicates the ene-diol as a key intermediate in the oxidation of oxyhaemoglobin by monosaccharides:

$$HOCH_2—CH(OH)—CHO \rightleftharpoons HOCH_2—C(OH)=CH(OH$$

Ene-diol

The autoxidation of the ene-diol-generated H_2O_2 has been described (Thornalley *et al.*, 1983*b*):



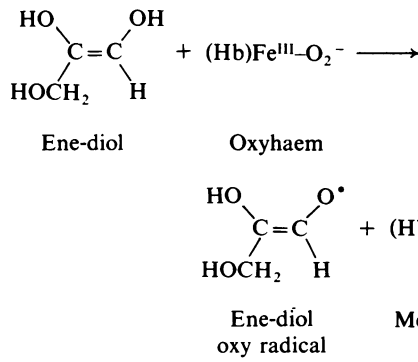
Ene-diol — Ene-diol oxy radical — Hydroxy-pyruvaldehyde

The H_2O_2 thereby produced is proposed to oxidize desoxyhaemoglobin in a Type III haemoglobin oxidation reaction (Wallace *et al.*, 1978):

$$(Hb)Fe^{II} + H_2O_2 \rightarrow \rightarrow \text{Methaemoglobin,}$$

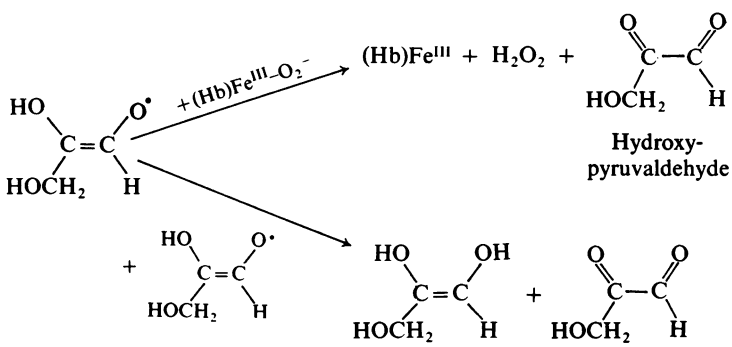
haemichrome and H_2O

This reaction with H_2O_2 appears to mediate approx. 58% of the oxidation of oxyhaemoglobin induced by autoxidizing monosaccharides. The remaining 42% of the oxidation reaction is H_2O_2-independent and is proposed as a Type II reductant (ene-diol)-mediated oxidation of oxyhaemoglobin:

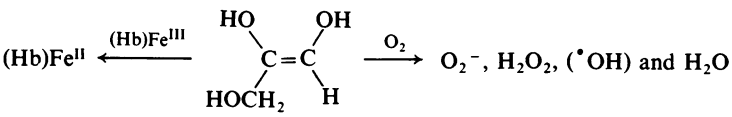


Ene-diol — Oxyhaem — Ene-diol oxy radical — Methaem

The ene-diol oxy radical may then disproportionate or react with further oxyhaemoglobin:



The rate of reduction of methaemoglobin by glyceraldehyde is increased by addition of DETAPAC, of superoxide dismutase, of catalase and of superoxide dismutase + catalase together, i.e. the rate of methaemoglobin reduction by glyceraldehyde is enhanced by co-ordinating trace metal ions with DETAPAC and by removing O_2^- and H_2O_2 from the reaction medium. These effects are consistent with the reduction of methaemoglobin and the reduction of O_2 (autoxidation) being competing fates for the ene-diol formed from glyceraldehyde under physiological conditions:



Methaem reduction Autoxidation

The non-enzymic reduction of methaemoglobin to oxyhaemoglobin has similarly been demonstrated for ascorbic acid (Tomada *et al.*, 1978) and several other physiological and pharmocological agents (Nichol *et al.*, 1968; reviewed by Kiese, 1974).

In the presence of 1mM-DETAPAC, carbonmonoxyhaemoglobin decreased the rate of O_2 consumption by 50mM-glyceraldehyde with 1mM-DETAPAC. This indicates that haemoglobin is interacting with the autoxidative process of glyceraldehyde by a mechanism other than the simple redox reaction between the haem groups and the ene-diol of glyceraldehyde. The globin may decrease the rate of glyceraldehyde autoxidation by scavenging free-radical intermediates in the autoxidative process. The aldehyde tautomer of glyceraldehyde may bind to available basic amino acid side chains (lysine and terminal valine residues) vis Schiff-base formation (Nigen & Manning, 1978; Acharya & Manning, 1980). These factors may account for the relatively high rate of glyceraldehyde consumption yet low rate of O_2 consumption observed in the glyceraldehyde/haemoglobin systems.

From results presented here, treatment of oxyhaemoglobin with high concentrations of monosaccharides is expected to result in the oxidation of haemoglobin to methaemoglobin and haemochromous material.

This work was supported by Grant HL19532 from the National Institutes of Health, by the Science and Engineering Research Council and by the Medical Research Council. The authors thank Dr. F. Landsberger (The Rockefeller University, New York, NY, U.S.A.) for the use of his e.s.r. facilities.

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