Experientia 40 (1984), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Short Communications

The autoxidation of glyceraldehyde and other simple monosaccharides

S. P. Wolff, M. J. C. Crabbe[1] and P. J. Thornalley[2, 3]

Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW (England), 15 March 1983

Summary. Simple monosaccharides are shown to autoxidize, under physiological conditions, forming dicarbonyl compounds and hydrogen peroxide via reactive intermediates of dioxygen and carbon centred free radicals. The rate of enolisation of the substrate to form an enediol is the rate limiting step of this spontaneous process.

The autoxidation of biological metabolites generates reactive intermediates[4], such as organic free radicals, superoxide and hydroxyl radicals, and induces oxidation of biological materials, manifest as thiol oxidation[5], lipid peroxidation[6] and enzyme inactivation[7]. These changes are symptoms of oxidative stress and pernicious to cellular systems. The unusually wide substrate specificity of an oxidoreductase, aldose reductase (aldehyde reductase B)[8], led us to consider the possibility that its substrates, all compounds containing the α-hydroxyketo group  , were susceptible to autoxidation. Our observations show that α-hydroxyketones do indeed autoxidize under physiological conditions, via the ene-diol tautomer, with the generation of carbon-centred free radicals, superoxide and hydroxyl radicals, producing hydrogen peroxide and α-dicarbonyl compounds. We suggest that the autoxidation of α-hydroxyketones should be considered along with the autoxidation of lipidic and other materials, as a factor to be implicated in oxidative stress and associated disease processes.

We can exemplify the general features and mechanisms of α-hydroxyketone autoxidation by reporting studies with DL-glyceraldehyde (GCHO). Its effects in vitro on several biological systems have been studied e.g. as a preferred substrate for aldose/aldehyde reductases[8], as an antisickling agent[9] and as an antitumor agent[10]; it has in addition, a biologically important-3-phosphoester and as a simple aldose it may model higher sugar autoxidative processes.

GCHO autoxidized under physiological conditions - as judged by GCHO and oxygen consumption (fig. 1, a and b) with concomitant production of hydroxy-pyruvaldehyde (HPA). This was the predominant product (50% of GCHO decrease) although some hydroxy-pyruvate (HPAA) was also detected. Ferricytochrome c was reduced by autoxidizing GCHO (fig. 1, b) but the rate of ferricytochrome c reduction was inhibited only 38% by addition of 300 units/ml copper-zinc superoxide dismutase. This suggests that superoxide is produced from autoxidizing GCHO along with other reductants of cytochrome c.

The temperature dependence of the rate of ferricytochrome c reduction by GCHO paralleled that of iodine uptake by GCHO (fig. 1, c). This uptake of iodine by α-hydroxyketones is characteristic of the ene-diol tautomer and the

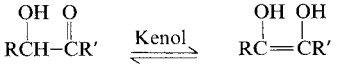
The reduction of ferricytochrome C

Substrate	Rate of reduction
Glyceraldehyde	100%
Glycolaldehyde	74%
Dihydroxyacetone	117%
β-Hydroxypyruvate	108%
Erythrose	32%
Propionaldehyde	0%
Acetaldehyde	0%
Pyruvate	0%
Glycerate	0%
Lactate	0%

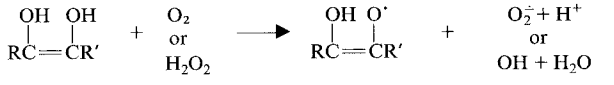
Rates relative to glyceraldehyde. All substrates 50 mM, pH 7.4 phosphate buffer.

Scheme. A mechanism for the autoxidation of α-hydroxyketones

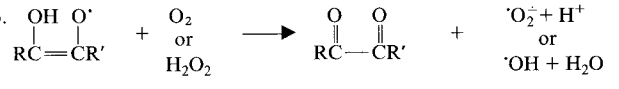
1. Enolisation



2. Autoxidation

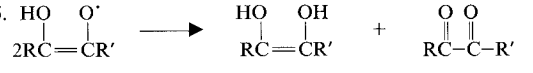


3.



4. $2O_2^{\cdot-} + 2H^+ \longrightarrow O_2 + H_2O_2$

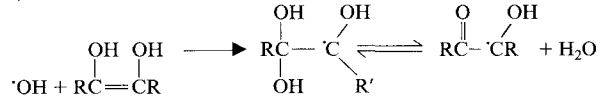
5.



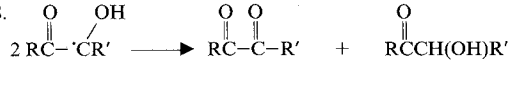
Hydroxyl radical quenching reactions

6. a) Abstraction:

7. b) Addition:



8.



a) R, R′ = alkyl or H.

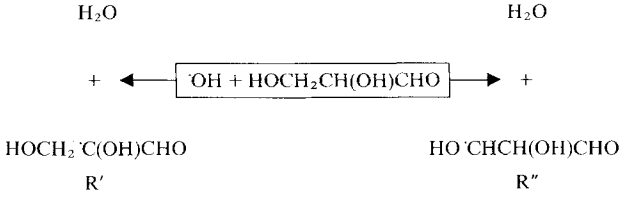
b) Ene-diol and mediating free radicals may be co-ordinated to trace metal ion catalysts.

observed parallel temperature-dependent behavior suggests that enolisation of GCHO is the rate determining step in the autoxidation process.

Arrhenius plots of the rates of iodine uptake and cytochrome c reduction by GCHO gave an estimate for the enolisation/autoxidation reaction of about 1 kJ/mol^{-1}. Figure 1, c shows that the rate of iodine uptake by GCHO accelerated as physiological temperatures were approached. Careful temperature control is, therefore, necessary if GCHO autoxidation is to be avoided.

Further evidence for the importance of the ene-diol tautomer came from examination of the propensity with which a range of ketone derivatives reduced ferricytochrome c. The table shows that only those compounds with the ability to form an ene-diol tautomer rapidly reduced ferricytochrome c. The ability of an α-hydroxyketone to enolise parallels the susceptibility of the α-hydroxyketone to autoxidation (Equation 1).

Free radical involvement in α-hydroxyketone autoxidation was investigated using the ESR technique of spin trapping with the spin trapping agent, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Figure 2, a shows the ESR spectrum produced by autoxidizing GCHO in the presence of DMPO at pH 8.6. The ESR spectrum can be resolved into 2 component spin adduct ESR spectra (DMPO-OH and DMPO-R) indicating production of hydroxyl (·OH) and a carbon-centred free radical (R·) respectively. Lowering the GCHO concentration 10-fold, gave an increase in DMPO-OH with a concomitant decrease in DMPO-R, indicating that R is formed from the reaction of hydroxyl radicals with GCHO.



Use of a more diagnostic spin trap, 2-methyl-nitrosopropane (TNP) showed both hydroxyalkyl radicals were produced with spin adduct concentrations of R′ and R″ present in the ratio R′:R″ 4:1 (R′ and R″ are not distinguished by DMPO) (data not shown).

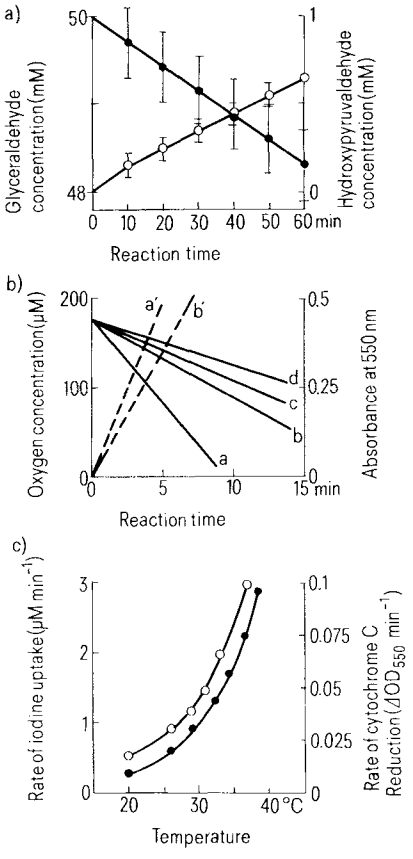


Figure 1. The Characterization of glyceraldehyde autoxidation. *a* Glyceraldehyde consumption/hydroxypyruvaldehyde production. GCHO, HPA and HPAA were identified by chromatography of reaction mixtures on silica gel 60 thin layer plates against authentic commercial GCHO (R_f=0.5), HPAA (R_f=0.25) and prepared[16] HPA (R_f=0.8); cf methyl glyoxal (R_f=0.92). The developing solvent was n-butanol (1): ethylacetate (10): c-propanol (6): acetic acid (3) water (1). 2,4-Dinitrophenylhydrazine (2,4-DNP) was used as the locating agent (spray of saturated 2,4-DNP in 2M HCl). GCHO was measured as the 2,4-DNP hydrazone[17] by removing the GCHO from the plate, resuspending the 2,4-DNP-GCHO in 2% diethanolamine in pyridine and measuring the absorbance at 360 nm, calibrated against known GCHO concentrations. HPA production was monitored as the Girard T reagent[18], and reduced glutathione-adducts[19]; the latter formed enzymatically in the presence of glyoxalase I[19].

b Oxygen consumption - measured in a Clark-type oxygen electrode with (a) control (50 mM GCHO), (b)+330 units/ml SOD, (c)+1 mM DETAPAC and (d)+250 units/ml CAT. Cytochrome c reduction, followed at 550 nm[20] with (a′) control (50 mM GCHO), (b′)+330 units/ml SOD.

c The temperature dependence of the rate of GCHO (12.5 mM) enolisation, measured by the rate of iodine uptake[11], and the rate of autoxidation, monitored by cytochrome c reduction[20].

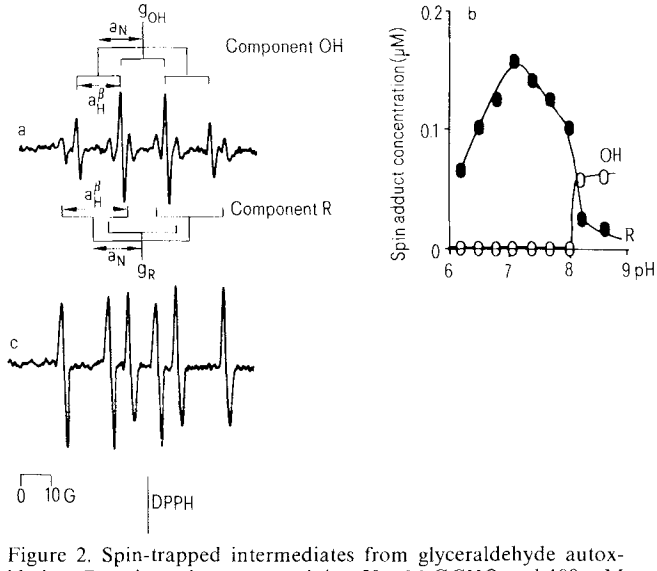


Figure 2. Spin-trapped intermediates from glyceraldehyde autoxidation. Reaction mixtures containing 50 mM GCHO and 100 mM DMPO were incubated in 50 mM sodium phosphate/pyrophosphate for 10 min at 37 °C then the ESR spectrum recorded on a Varian E104, X band ESR spectrometer with an E935 data acquisition system. Spin adduct concentrations were computed from a calibrated double integral. Instrumental details; field set 3385G, field scan 100G, modulation frequency 100 kHz, modulation amplitude 1.0G, receiver gain 1×10^4, time constant 0.128 s microwave power 10 mW, microwave frequency 9.478GHz. Buffer systems were sodium phosphate pH 6–8.2; pyrophosphate pH 8.4–9. DMPO-R = 5,5-dimethyl-2-hydroxylalkyl pyrrolidino-1-oxyl. DMPO-OH = 5,5-dimethyl-2-hydroxypyrrolidino-1-oxyl. a, pH 8.6; b, data obtained from calibrated double integrals, pH 6–9; c, pH 7.4.

Consistent with this is the observation that bovine catalase (250 units/ml) inhibited the formation of both components whereas copper-zinc superoxide dismutase (330 units/ml) enhanced the formation of both components and indicates that hydrogen peroxide is a precursor of the hydroxyl radical. Autoxidising GCHO therefore produces 1-hydroxylalkyl (R ·) and hydroxyl (· OH) radicals at pH 8.6.

The free radical production from GCHO, as judged by spin trapping, showed a peculiar pH profile (fig. 2, b): below pH 8.2, only DMPO-R was observed, figure 2, c. This is consistent with a pKa of 8.2 for the ene-diol of GCHO. Above this pH the ene-diolate anion predominates and rapidly reduces dioxygen by a redox mechanism (Equations 2+3); the ene-diol is short-lived. Below pH 8.2, the unionized ene-diol predominates, autoxidation is slower and hydroxyl radicals produced are scavenged by a free radical addition mechanism (Equation 7). Hydroxyl radicals are also scavenged by the free aldehyde throughout the pH range (Equation 6). Both ene-diol oxy radicals $\overset{O}{\underset{|}{R}}\!C{=}\overset{O^{\cdot}}{\underset{|}{C}}R'$ and superoxide radicals $O_2\cdot$ are included in the mechanism of α-hydroxyketone autoxidation (scheme) yet these radicals were not observed by spin-trapping. This can be expected from the relatively low sensitivities of detection by spin-trapping for these radicals[12,13]. Evidence of ene-diol oxy radical intermediates comes from the ESR spectrum of α-hydroxyketones at high pH, under anaerobiosis[14]. Superoxide involvement was indicated indirectly; superoxide dismutase decreased the rate of cytochrome c reduction (38%) and oxygen consumption (40%) and stimulated DMPO-R and DMPO-OH production from GCHO. A generalized, overall mechanism for α-hydroxyketone autoxidation is given in the scheme. The ene-diol oxy and R radicals may disproportionate (Equations 5 and 8) to a keto/enol reactant and an α-dicarbonyl product molecule. Products from the overall reaction, α-dicarbonyl compounds and hydrogen peroxide are both potentially toxic to biological systems. Low levels of hydroxypyruvaldehyde-3-phosphate have been detected in erythrocytes[15]. The addition of metal ion chelators to autoxidizing GCHO reduced the rate of oxygen consumption by about 25% (fig. 1, a), suggesting that trace metal ions may catalyze α-hydroxyketone autoxidation, although their involvement does not appear to be obligatory. Metal ions may co-ordinate to cis-ene diol and ene-diol-oxy radicals to facilitate electron transfer to dioxygen.

Our observations suggest that α-hydroxyketones become susceptible to autoxidation at physiological temperatures, albeit dependent on the availability of the ene-diol tautomer. In many systems, this has not been hitherto appreciated. We suggest that phenomena observed in some experimental systems using α-hydroxyketones may not be ascribed to the substrate but to the intermediates and products of their autoxidation processes.

1 To whom correspondence should be addressed.
2 Present address: Biophysical Chemistry Department, Paterson Laboratories, Christie Hospital, Manchester, M20 9BX, G.B.
3 Acknowledgments. We should like to thank the NIH, MRC ICI Pharmaceuticals Ltd and the SERC for financial support; Dr D. Earle for helpful conversations, Mr. A.J. Bron for provision of laboratory facilities and Dr. J.V. Bannister for the generous gift of superoxide dismutase.
4 Fridovich, I. A. Rev. Biochem. *44* (1975) 147.
5 Torchinskii, Y.M. Sulphur in proteins. Pergamon Press, Oxford 1981.
6 Bus, J.S., and Gibson, J.E., in: Reviews in Biochemistry and Pharmacology, p. 125. Eds. E. Hodgson, J.R. Bend and R.M. Philpot. Elsevier North Holland, New York 1979.
7 Armstrong, D.A., and Buchanan, J.D., Photochem. Photobiol. *28* (1978) 743.
8 Crabbe, M.J.C., Ting, H-H. and Basak Halder, A., in: Enzymology of carbonyl metabolism, p. 329. Eds. H. Weiner and B. Wermuth. Alan Liss Inc., New York 1982.
9 Nigen, A.N., and Manning, J.M., Proc. natl Acad. Sci. USA *74* (1977) 367.
10 Schauenstein, E., Esterbauer, H., and Zollner, H., in: Aldehydes in Biological systems, p. 103. Pion Ltd, London 1977.
11 Hall, A., and Knowles, J.R., Biochemistry *14* (1975) 4348.
12 Finkelstein, E., Rosen, G.M., and Rauckman, E., Archs Biochem. Biophys. *200* (1980) 1.
13 Perkins, M.J., Adv. Phys. org. Chem. *17* (1980) 1.
14 Isbell, H.S., Adv. Chem. Ser. *117* (1973) 70.
15 Cogoli-Greuter, M., and Christen P., J. biol. Chem. *256* (1981) 5708.
16 Reeves, H.C., and Ajl, S.J., J. biol. Chem. *240* (1965) 569.
17 Johnson, P., Critchfield, F.E., and Ruch, J.E., Analyt. Chem. *34* (1962) 1389.
18 Mitchel, R.E.J., and Birnboim, H.C., Analyt. Biochem. *81* (1977) 47.
19 Bergmeyer, H.U., Oawehn, K., and Grassl, M., in: Methoden der Enzymatischen Analyse, vol 1, 3rd edn., p. 454. Ed. H.U. Bergmeyer. Verlag Chemie, Weinheim 1974.
20 Fridovich, I., J. biol. Chem. *245* (1970) 4053.

0014-4754/84/030244-03$1.50 + 0.20/0

C-18 Hydroxy steroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*[1]

G. Cimino, S. De Rosa, S. De Stefano and G. Sodano

Istituto per la Chimica M.I.B., Via Toiano 6, I-80072, Arco Felice, Naples (Italy), 28 April 1983

Summary. Five new polyoxygenated steroids, 24-methylcholesta-1,4,22E-trien-16β,18,20ξ-triol-3-one (**1**), cholest-1,4,22E-trien-16β,18,20ξ-triol-3-one (**2**), 24-methylcholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**3**), cholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**4**) and 27-nor-24-methylcholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**5**), have been found in a marine organism, the gorgonian *Leptogorgia sarmentosa*.

The polyoxygenated steroids are largely present in the secondary metabolism of marine organisms[2] but they rarely show a hydroxy group at C-18. A pregnane derivative with this structural feature has been reported[3], together with its C-18 acetate, from the telestacean octocoral *Telesto riisei*, while another C-18 acetate derivative has been found[4] in the hydroid *Eudendrium*.

Continuing our researches on the steroids of Mediterranean gorgonians[5–7] we have now found in *Leptogorgia sarmentosa* a series of steroids (**1–5**) characterized by oxidation patterns which include a C-18 hydroxy group.

The gorgonian was extracted with acetone at room temperature. The extract, after concentration in vacuo, was partitioned between diethyl ether and water. The ether-