ENERGY-LINKED OXIDATION OF GLUTARALDEHYDE BY RAT LIVER MITOCHONDRIA

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1. Introduction

Through its property of forming cross links, the bifunctional reagent glutaraldehyde is of value in studies of the conformation and enzymic activities of proteins in the crystalline and dissolved states. It reacts, probably in polymeric α,β-unsaturated aldehyde forms [1], in aqueous solution at neutral pH values, and mainly with the ϵ-amino groups of lysine residues [2]. It is also used as a fixative in electron microscopic studies of mitochondria. With isolated mitochondria [3,4] and chloroplasts [5], it is possible, by controlling the extent of cross linkage produced by glutaraldehyde, to achieve fixation of gross ultrastructure while preserving electron transport and functional activities such as electron transport-dependent ion translocation.

During such experiments on controlled fixation by glutaraldehyde, it was found that this substance is oxidized quite rapidly by rat liver mitochondria. The

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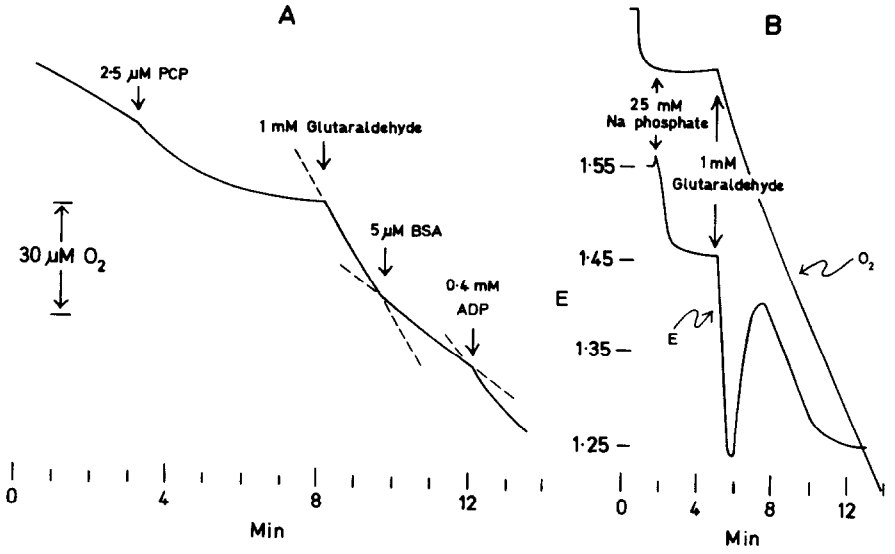

Fig. 1. Glutaraldehyde oxidation and mitochondrial swelling. Total volume, 4.0 ml; 1.5 mg of mitochondrial protein/ml. The reaction mixture contained initially: in A, 125 mM sucrose, 0.5 mM EDTA, 5 mM $MgCl_2$ and 5 mM sodium phosphate pH 7.8; in B, 121 mM sucrose and 0.5 mM EDTA. Other additions as shown by arrows; phosphate, pH 7.8; BSA, crystalline bovine plasma albumin. Temperature 22°C.

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oxidation is energy-linked; thus it is stimulated by ADP and uncoupling agents and inhibited by oligomycin, and it also drives oscillatory mitochondrial volume changes. It is much less inhibited by high sucrose concentrations than is the oxidation of the usual anionic substrates. Formaldehyde and acetaldehyde are also oxidized, but at considerably lower rates.

2. Materials and methods

Rat liver mitochondria were isolated, and washed twice, in 0.44 M sucrose containing 1 mM EDTA pH 7.8. For respiratory studies they were incubated in a square glass cell maintained at 20°C or 22°C; O_2 uptake, measured polarographically with a Clark electrode (Yellow Springs Instrument Co., Ohio, USA), and changes in optical extinction (E), taken as an indication of swelling or contraction, were recorded simultaneously. The light was passed through a filter which transmitted wavelengths above 650 mμ, and use of a logarithmic converter [6] enabled extinctions to be recorded on a linear scale (fig. 1B). Redistilled glutaraldehyde was obtained, as a 0.8 M aqueous solution stored in ampoules under N_2, from Polysciences, Inc., Warrington, Pennsylvania, USA; other compounds were purchased from usual sources.

Table 1
Mitochondrial respiration and swelling with aldehydes and other substrates, at two osmolarities.

	O_2 uptake (μM/min)		$-\frac{dE}{dt}\frac{100}{E}$ (%/min)	
Sucrose concentration (M)	0.12	0.44	0.12	0.44
Formaldehyde				
(1 mM)	11	5	5	0.1
(3.5 mM)	8*	5*	4	0.1
Acetaldehyde				
(1 mM)	10	4	5	0.1
(3.5 mM)	15	6	8	0.1
(25 mM)	9*	9*	23	0.6
Glutaraldehyde				
(1 mM)	26	20	18	2.4
Succinate				
(1 mM)	37	4	20	0.1
(5 mM)	63	20	35	0.7
Glutamate				
(1 mM)-malate (1 mM)	16	2	7	0.0
Ascorbate				
(5 mM)-TMPD (0.2 mM)	60	61	16	5.5

Conditions as in fig. 1B, the rates being those after addition of P_i. Respiration calculated for 2 mg of protein/ml. Temperature 20°C. Acids were brought to pH 7.8 with NaOH.
* Initial rate: respiration decreased rapidly.

3. Results and discussion

3.1. *Oxidation of aldehydes*

In experiments under phosphorylating conditions (fig. 1A), removal of endogenous substrate was expedited by addition of pentachlorophenol (PCP). When respiration had virtually ceased, addition of glutaraldehyde caused a rapid O_2 uptake. On removal of the PCP by plasma albumin [7], the respiration was inhibited, and increased again after the addition of ADP. Glutaraldehyde oxidation thus seems to be under respiratory control and to be coupled with phosphorylation. Consistent with this, it is inhibited by oligomycin (1 μg/mg of protein), with release by dinitrophenol. It is completely inhibited by antimycin and rotenone (1 and 0.7 μg/mg of protein, respectively) and so presumable involves an NAD- or NADP-dependent dehydrogenase.

Under the appropriate conditions [8], glutaraldehyde drives the oscillatory volume changes (fig. 1B) associated with ion translocation (the light scattering is known to be a measure of the mitochondrial volume [4]). Table 1 shows respiratory and swelling rates with various substrates under oscillatory conditions. Respiration with glutaraldehyde is about two-thirds of that with succinate at the same concentration; formaldehyde and acetaldehyde give lower rates. Whereas oxidation of the usual anionic substrates is greatly inhibited by high sucrose concentration [9], that of glutaraldehyde and the other aldehydes is much less affected. This is probably because sucrose inhibits the anion carriers of the inner membrane [10] and oxidation of aldehydes and tetramethyl-*p*-phenylenediamine (TMPD) will not depend on these. The swelling rates run roughly parallel to the respiration (table 1); they are much lower with mitochondria in 0.44 M

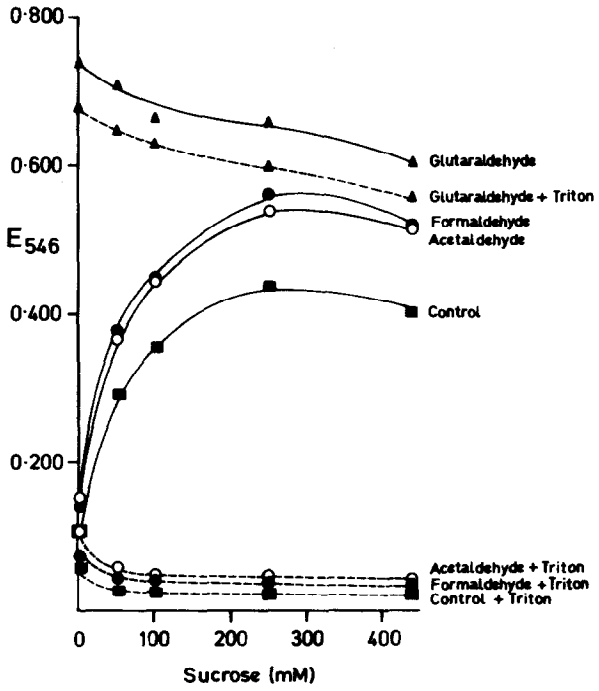

Fig. 2. Test for fixation of mitochondria by aldehydes. The mitochondria were suspended, at a concentration of 20 mg of protein/ml, in preparation medium (0.44 M sucrose – 1 mM EDTA) containing 50 mM aldehyde, and left for 15 min at room temperature. 0.1 ml aliquots were then diluted 40-fold with sucrose solutions to give the final molarities shown in the figure. Ten min later the extinction at 546 mμ was measured in a Unicam SP 500 spectrophotometer. Then 0.02% Triton X-100 was added to each sample and after another 10 min the extinctions were measured again.

than in 0.12 M sucrose, but it is clear that high sucrose concentrations have less effect with glutaraldehyde and TMPD than with the anionic substrates.

3.2. *Fixation of mitochondria by aldehydes*

The finding that mitochondria oxidize low concentrations of glutaraldehyde prompted us to investigate the mitochondria-fixing capabilities of this and other aldehydes. Prevention of osmotic volume changes and of swelling or lysis caused by Triton X-100, as judged from extinction measurements (fig. 2), was used as an indication of fixation. Clearly, even at a concentration (50 mM) far above the substrate range, formaldehyde and acetaldehyde do not prevent osmotic or Triton swelling whereas the bifunctional glutaraldehyde is effective under these conditions. Other experiments [11] have established that the lowest concentration at which glutaraldehyde will fix mitochondria by the above criteria is about 5 mM, whereas 1 mM is insufficient at 20°C and with protein concentrations of 1 to 2 mg/ml.

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References

[1] F.M.Richards and J.R.Knowles, J. Mol. 37 (1968) 231.
[2] A.F.S.A.Habeeb and R.Hiramoto, Arch. Biochem. Biophys. 126 (1968) 16.
[3] K.Utsumi and L.Packer, Arch. Biochem. Biophys. 121 (1967) 633.
[4] L.Packer, J.M.Wrigglesworth, P.A.G.Fortes and B.C. Pressman, J. Cell Biol. 39 (1968) 382.
[5] L.Packer, J.M.Allen and M.Starks, Arch. Biochem. Biophys. 128 (1968) 142.
[6] A.G.Marr and L.Marcus, Anal. Biochem. 2 (1961) 576.
[7] E.C.Weinbach and J.Garbus, J. Biol. Chem. 241 (1966) 169.
[8] L.Packer, K.Utsumi and M.G.Mustafa, Arch. Biochem. Biophys. 117 (1966) 381.
[9] D.Johnson and H.Lardy, Nature 181 (1958) 701.
[10] J.B.Chappell and B.H.Robinson, personal communication.
[11] L.Packer, J.K.Pollak, E.A.Munn and G.D.Greville, in preparation.