PREPARATION OF DIHYDROLIPOAMIDE BY ELECTROLYTIC REDUCTION

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1. Introduction

Lipoic acid plays a central role in the action of the α-oxoacid dehydrogenase enzyme complexes. Enzyme-bound in the form of lipoamide it participates in a cyclic series of reactions comprising reductive acylation, acyl transfer to coenzyme A and reoxidation by NAD^+. The individual enzymes are also active with externally-supplied, unbound, lipoamide and studies of these reactions sometimes require the use of the reduced form, dihydrolipoamide, in which the disulphide bond of lipoamide has been reduced to the dithiol form. Reduction is generally achieved by treatment with sodium borohydride followed by extraction into benzene or chloroform and subsequent removal of the solvent by distillation or evaporation in vacuo [1–3].

In the course of investigations on the regulation of a bacterial α-oxoglutarate dehydrogenase [4] we required dihydrolipoamide to study the dihydrolipoamide dehydrogenase component of the complex and devised a simple electrolytic procedure for its production from lipoamide. This communication presents the details and advantages of the procedure.

2. Experimental

The technique employed follows that previously used for the electroreduction of disulphide bonds in proteins [5]. The apparatus used is illustrated in fig. 1. The cathode compartment, A, is a small rimless beaker (about 40 ml capacity) fitted with a side-arm and contains a pool of mercury (Analar grade) the surface of which can be stirred with a small magnetic

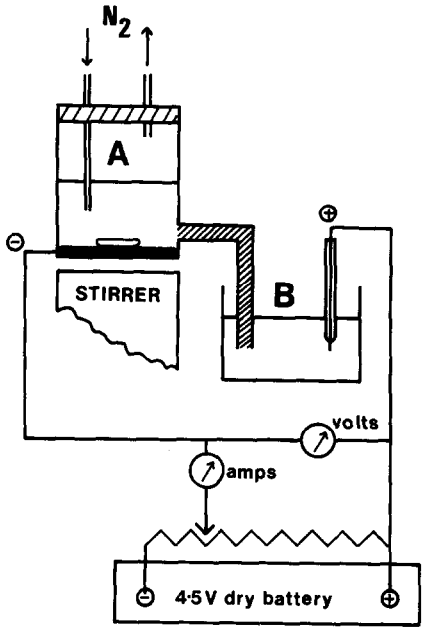

Fig. 1. Apparatus for electrolytic reduction. See text for explanation.

'flea'. A piece of platinum wire is fused into the side of the beaker and makes contact with the mercury. The solution to be electrolysed sits over the mercury pool and electrical contact between this solution and the anode compartment, B, (a platinum electrode dipping into saturated KCl solution) is effected through the side-arm which contains 2% agar gel in saturated KCl. The cathode compartment is fitted with a stopper through which two syringe needles pass; these allow nitrogen to be flushed through the solution being electrolysed. The source of the potential is a dry

battery connected to the two electrodes via a variable potentiometer; both the potential and the current may be monitored with suitable meters.

A 20 mM solution of oxidised lipoamide (Sigma) in ethanol was first prepared and diluted to 1 mM with 0.1 M Tris–HCl, pH 8.0. Ten ml of this solution were introduced into the electrolysis cell over the mercury pool, stirred continuously and de-oxygenated for a few minutes by a stream of O_2-free nitrogen. A potential of −3 V was then applied to the mercury pool cathode and the progressive decrease in current was followed. At intervals, 0.05 ml portions were withdrawn and added to a spectrophotometer cuvette containing 0.93 ml of 0.1 M Tris–HCl, pH 8.0 and 0.02 ml of 10 mM 5,5′-dithiobis-(2-nitrobenzoate); after mixing the absorbance at 412 nm was measured. On the basis of a molar absorbance of 13 600 $1 \cdot mole^{-1} \cdot cm^{-1}$ for the yellow thionitrobenzoate ion [6] the thiol content and state of reduction of the lipoamide were determined.

Polarographic measurements on oxidised and reduced lipoamide were made with a Beckman Electroscan 30 recording polarograph using a dropping mercury cathode and a saturated calomel reference anode.

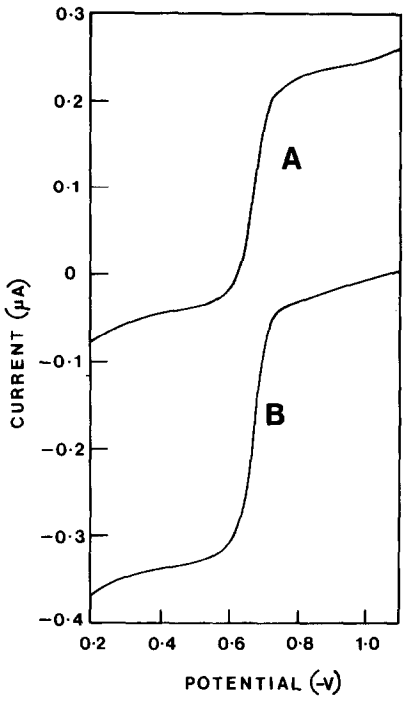

Fig. 3. Polarographic waves of oxidised and electroreduced lipoamide. Measurements were made on 40 μM lipoamide in 0.1 M Tris–HCl, pH 8.0. A, oxidised lipoamide; B, dihydrolipoamide. Current compensation of 0.25 μA/V was applied.

3. Results and discussion

Typical progress curves for the electroreduction of lipoamide are shown in fig. 2. After 30 min of electrolysis, both the current and thiol content reached limiting values corresponding to total reduction of the lipoamide disulphide bond to two thiol groups. Reduction was also accompanied by a complete change of polarographic activity (fig. 3). Whereas the oxidised lipoamide gave rise to a cathodic reduction wave due to the disulphide bond, the dihydrolipoamide showed no cathodic wave but only an anodic oxidation wave due to its thiol groups. Titration with *N*-ethylmaleimide resulted in progressive destruction of this anodic wave and gave an end-point again corresponding to two thiol groups per molecule of dihydrolipoamide.

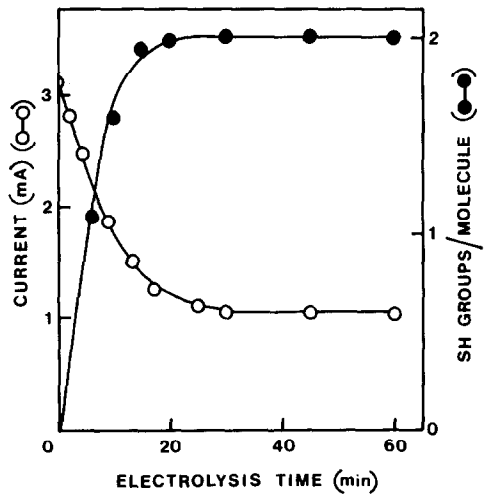

Fig. 2. Time course of the electrolytic reduction of lipoamide.

It is important to note that although the reduction is carried out over a pool of mercury, no interference with the dihydrolipoamide is thereby created. The fact that reactions with 5,5′-dithiobis-(2-nitrobenzoate) and *N*-ethylmaleimide indicated quantitative reduction to the dithiol confirms that no mercury mercaptide formation occurs. Moreover, the activity of the dihydrolipoamide with dihydrolipoamide dehydroge-

nase [4] indicates that only the disulphide bond is attacked during electrolysis.

The polarographic activity of lipoic acid and its reduced form was demonstrated some years ago [7–9] but, to our knowledge, the electroreducibility which it suggests has not been utilized for the preparation of dihydrolipoamide. The electrolytic method offers the advantages of being simple, inexpensive, rapid and quantitative. No excess reducing agent needs to be removed at the end of the reduction and the dihydrolipoamide is usable without further treatment or purification. These features make the method of particular value in investigations of lipoamide-linked enzyme systems.

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References

[1] Gunsalus, I. C., Barton, L. S. and Gruber, W. (1956) J. Amer. Chem. Soc. 78, 1763–1766.
[2] Wagner, A. F., Walton, E., Boxer, G. E., Pruss, M. P., Holly, F. W. and Folkers, K. (1956) J. Amer. Chem. Soc. 78, 5079–5081.
[3] Reed, L. J., Koike, M., Levitch, M. E. and Leach, F. R. (1958) J. Biol. Chem. 232, 143–158.
[4] Parker, M. G. and Weitzman, P. D. J. (1973) Biochem. J. 135, 215–223.
[5] Cecil, R. and Weitzman, P. D. J. (1964) Biochem. J. 93, 1–11.
[6] Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70–77.
[7] Reed, L. J., Gunsalus, I. C., Schnakenberg, G. H. F., Soper, Q. F., Boaz, H. E., Kern, S. F. and Parke, T. V. (1953) J. Amer. Chem. Soc. 75, 1267–1270.
[8] Ke, B. (1957) Biochim. Biophys. Acta 25, 650–651.
[9] Sponar, J. and Jirsa, M. (1958) Biochim. Biophys. Acta 29, 434–435.