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Reversible reduction of thioctamide catalyzed by the α-ketoglutaric dehydrogenase complex

The pyruvic and α-ketoglutaric (KG) dehydrogenases of mammalian tissues are complex units of high molecular weight ($4 \cdot 10^6$ and $2 \cdot 10^6$ g/mole respectively)[1,2]. Under the electron microscope*, the latter appears as an essentially spherical particle of 120 A diameter which is consistent with the molecular weight calculated from sedimentation and diffusion constants. Both of these enzyme complexes contain tightly-bound, non-dialyzable 6,8-thioctic acid (α-lipoic acid) or a derivative of it active in the microbiological assay[2,3]. Direct evidence that thioctic acid functions in the oxidation of α-ketoacids has been obtained from studies on bacterial preparations[4,5]. One of the intermediate steps postulated in the sequence of reactions is the oxidation of dithioloctanoate by DPN (Reaction 1)

$$T(SH)_2 + DPN^+ \rightleftarrows TS_2 + DPNH + H^+ \qquad (1)$$

The presence of this enzymic activity in purified fractions from *E. coli* has been demonstrated by coupling it to the reduction of pyruvate with lactic dehydrogenase and measuring the decrease in $-SH$[6].

We have now found that the KG dehydrogenase purified from hog hearts[2] catalyzes the reversible oxidation of reduced DPN (DPNH) by 6,8-thioctate and by 6,8-thioctamide**. The reaction can be demonstrated readily in both directions by measuring the absorption of DPNH at 340 mμ (Fig. 1). (+) Thioctate is active while (—) thioctate is inactive in the reaction. The presence of the inactive isomer had no effect on the velocity of the reaction with the (+) isomer.

There are significant differences in the activity with thioctate and thioctamide. The pH optimum for the reaction with thioctamide is 7.1, which is about the same as for KG oxidation by DPN[7]. With thioctate as substrate, the activity increases sharply on decreasing the pH from 7.0 to 6.0. The concentration for half-maximal velocity with DL-thioctamide is $6 \cdot 10^{-4} M$, while maximal rates are not obtained with DL-thioctate even at $6 \cdot 10^{-3} M$. The rates of KG oxidation and of DPNH oxidation by thioctate or thioctamide are shown in Table I. It is seen that the partial reactions proceed at a faster rate than the over-all KG oxidation. The fact that, compared with the reduction of thioctic acid, the reaction with thioctamide has a lower K_m and a pH optimum closer to that of KG dehydrogenase tentatively suggests that the natural bound cofactor

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might be closer in structure to thioctamide than to thioctic acid. It must be recognized that the reactions with thioctic acid and amide are probably mediated through the bound cofactor.

TABLE I

COMPARATIVE RATES OF REACTIONS CATALYZED BY KG DEHYDROGENASE

	μmoles/min/mg protein
KG oxidation	3.5
Thioctamide reduction	16.5
Thioctate reduction	4.6

KG oxidation was measured at pH 7.4 under conditions previously described[7] and thioctamide reduction as in Fig. 1 except that the substrate concentration was $2 \cdot 10^{-3} M$. For line 3, $4.5 \cdot 10^{-3} M$ DL-thioctate at pH 6.1 was used (sub-optimal conditions).

The equilibrium constant of the reaction has been measured at pH 7.1 and 23°. The average value of several determinations for

$$K = \frac{[TS_2]\,[DPNH]\,[H^+]}{[TS_2H_2]\,[DPN]}$$

was $1.65 \cdot 10^{-8}$. The E_0' for the thioctic acid couple calculated from the K was —0.301.

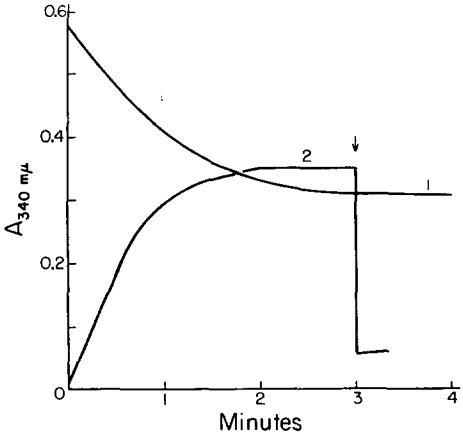

Fig. 1. Reversible reduction of thioctamide. Curve 1: The reaction mixture contained 100 μmoles phosphate buffer, pH 7.0, 0.29 μmoles DPNH and 2.3 μmoles DL-thioctamide in 2.5 ml. Curve 2: 100 μmoles phosphate, 1.66 μmoles DPN and 1.2 μmoles DL-dithioloctamide in 2.5 ml. The reaction was initiated by the addition of 12 μg and 25 μg respectively of KG dehydrogenase. At 3 min 0.01 ml lactic dehydrogenase and 1 μmole pyruvate were added to 2.

The diaphorase-like activity associated with purified α-ketoglutaric dehydrogenase[2,8] might be mediated through the reduction of the bound thioctic cofactor by DPNH since dithioloctanoate and dithioloctamide are readily oxidized in the presence of air and methylene blue.

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