OXIDATION OF α-KETOGLUTARATE BY ARTIFICIAL ELECTRON ACCEPTORS*

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The oxidation of pyruvate and α-ketoglutarate catalyzed by the respective dehydrogenase preparations (Jagannathan and Schweet, 1952; Hager, 1953; Sanadi and Littlefield, 1952) with non-physiological oxidants like $Fe(CN)_6^{-3}$ or dyes (Reaction 1) is independent of Coenzyme A (CoA) or diphosphopyridine nucleotide (DPN). Low levels of arsenite or Cd^{++} do not inhibit the oxidation of α-ketoglutarate by these compounds (Sanadi, Langley and White, 1959). On the other hand, the oxidation of pyruvate by ferricyanide

$$R\text{-}CO\text{-}COOH + Dye + H_2O \longrightarrow R\text{-}COOH + Dye\ H_2 + CO \qquad (1)$$

in thioctic (lipoic) acid deficient cells of Streptococcus faecalis is stimulated greatly by the addition of thioctic acid. The role of thioctate in Reaction 1 has been re-examined in view of the conflicting data.

$$R\text{-}CO\text{-}COOH + DPN^+ + CoASH \longrightarrow R\text{-}CO\text{-}S\text{-}CoA + DPNH + H^+ + CO_2 \qquad (2)$$

Table I shows that α-ketoglutaric dehydrogenase complex, when incubated with arsenite or Cd^{++} under reducing conditions, forms a derivative which is inactive in DPN reduction (Reaction 2) (Sanadi, Langley and White, 1959) but retains almost full activity in the reduction of 2,6-dichlorophenol indophenol. The participation of thioctate in the dye reduction seems highly improbable.

Preparations of α-ketoglutaric dehydrogenase kept frozen for several weeks generally show a decline in the DPN reduction activity and

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Table I

Additions	Δ A/min.	
	DPN (340 mμ)	Dye (600 mμ)
None	0.360	0.140
DPNH	0.360	0.160
Arsenite	0.324	0.131
DPNH + Arsenite	0.144	0.140
None	0.330	0.103
DPNH	0.309	0.101
Cd^{++}	0.270	0.099
DPNH + Cd^{++}	0.017	0.090

1.91 mg. of α-ketoglutaric dehydrogenase was incubated in 0.4 ml. for 3 min. at 30°C with 1.0 mg. albumin. The other components, as specified, were 0.4 μmole arsenite, 0.2 μmole cadmium chloride, 0.06 μmole DPNH and 25 μmoles phosphate at pH 6.8 with arsenite or 20 μmoles tris (hydroxymethyl) aminomethane at pH 7.4 with Cd^{++}. Aliquots of 0.02 ml. and 0.04 ml. respectively were assayed in the DPN reduction and dye reduction assays (Sanadi, Langley and White, 1959).

eventually coagulate. Table II shows that aged preparations, unlike fresh preparations (see Table I), are inhibited approximately 50% in the dye reduction assay by DPNH. Arsenite or α-ketoglutarate, if added before DPNH, afford considerable protection. Similar inhibition by DPNH is observed also in the DPN reduction assay (data not shown).

These results cast a slightly different light on the initial steps of the current concept of the steps involved in α-keto acid oxidation and may be explained on the basis of the following hypothetical scheme. DPT and TS_2 represent diphosphothiamine and thioctic acid respectively.

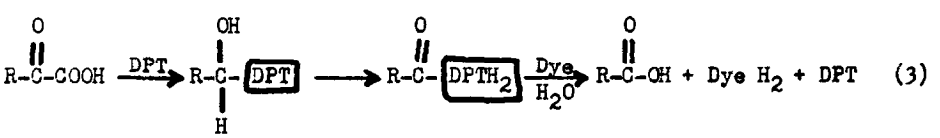
The aldehyde-DPT complex may exist as a resonance hybrid with acyl-reduced DPT which can be oxidized by ferricyanide or dyes with the simultaneous hydrolysis of the acyl group. In the physiological reaction the intermediate

Table II

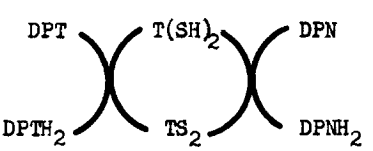
Additions	$\Delta_{600\ m\mu}$/min.	Inhibition %
None	0.090	
DPNH	0.046	49
Arsenite	0.092	-2
Arsenite, then DPNH	0.081	10
DPN	0.094	-4
None	0.110	
DPNH	0.052	53
DPNH, then arsenite	0.068	38
Arsenite, then DPNH	0.087	21
α-KG	0.110	0
α-KG, then DPNH	0.087	21

Conditions as in Table I except 0.04 ml. aliquot was assayed. One μmole α-ketoglutarate and 0.06 μmole DPN were in the preincubation mixture where indicated.

may react with thioctic disulfide to form acyl-s-thioctate as postulated earlier (Gunsalus, 1954; Reed, 1957). Reduction of the DPT by DPNH (Reaction 4) may prevent the primary condensation with the keto acid and result in inhibition of dye reduction. If this reduction of DPT occurs through thioctate, arsenite or Cd^{++} would be active inhibitors and would keep the DPT in the oxidized form and permit the oxidation by the dye to occur without change. Once the reduction has occurred, however, arsenite cannot reverse



(3)



(4)

it. Another way of preventing the reduction is by the addition of the α-keto acid which would yield the aldehyde-DPT intermediate and prevent formation of the enzymatically inactive $DPTH_2$.

Lipmann (1936) pointed out, on the basis of the chemical reduction of thiamine, that DPT could conceivably function as an oxidation reduction coenzyme. No evidence, however indirect, for such a role in an enzyme reaction has been observed previously.

References

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