HIGH ACTIVITY OF DPN LINKED ISOCITRATE DEHYDROGENASE IN MITOCHONDRIA FROM VARIOUS ORGANS

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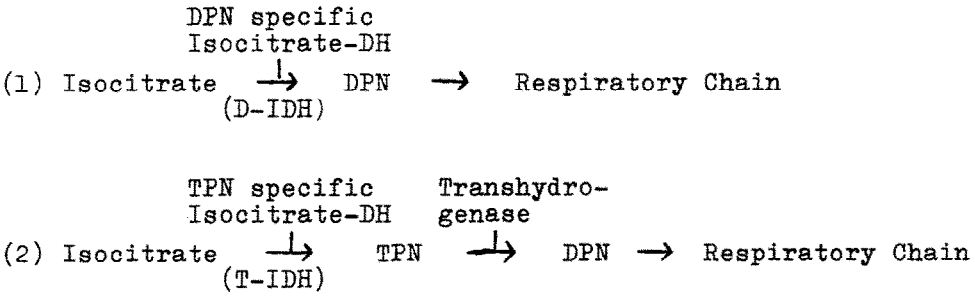
Two pathways for the oxidation of isocitrate in the tricarboxylic acid cycle have been discussed:



The relative importance of these alternative pathways was a matter of some controversy in particular for rat liver mitochondria. Kaplan et al. (1956), Purvis (1958), Stein, Kaplan et al. (1960) concluded that isocitrate is only oxidized by pathway (2). In contrast, Ernster et al. (1956, 1957, 1960) stated that the DPN linked route (1) can account for the full rate of isocitrate oxidation. Vignais et al. (1961) and Hawtrey (1962) suggested that both pathways are of about equal importance. The occurence of the D-IDH catalyzed way (1) was indicated also for heart (Plaut and Plaut, 1952), for brain and kidney (Vignais et al. 1961) and for certain tumor tissues (Hawtrey, 1962). However, most authors assumed that the TPN linked pathway is the normal, generally distributed one, whereas the DPN linked sequence occurs in special cases.

In this paper, evidence will be presented that mitochondria from all investigated animal organs contain a high activity

of DPN linked isocitrate dehydrogenase (D-IDH). In the mitochondria of some tissues the activity of the DPN specific enzyme may several fold surpass that of the TPN linked IDH. On this basis it is concluded that in all mitochondria the DPN linked pathway (1) plays an important role and may even be the major route for isocitrate oxidation. In the following paper, more precisely a separate role for the DPN and TPN linked enzymes in isocitrate oxidation will be defined on the basis of relating the enzyme activity to the content of respiratory enzymes in the mitochondria.

Experimental procedure

The D-IDH was extracted from mitochondria and assayed according to procedures described in detail elsewhere (Goebell and Klingenberg, 1963 a). These procedures include the presence of reduced glutathione and ADP in the extraction medium for stabilisation of the enzyme. The activity of D-IDH was measured under optimal conditions, such as defined by the concentrations of DPN, isocitrate, pH etc. and taking into account the specific activation of D-IDH by ADP (Goebell and Klingenberg, 1963 a). T-IDH was assayed under standard conditions described elsewhere (Delbrück et al., 1959).

Results

The enzyme activity of D-IDH extracted from mitochondria of various tissues as referred to the mitochondrial protein is given in table 1. For comparison the activity of the T-IDH has been measured in the same mitochondrial extracts. A considerable activity of D-IDH is found in the mitochondria of all investigated organs of the rat, the pigeon and the thoracic muscle of locusta migratoria. There are some variations with a higher activity in the insect muscle and the skeletal muscle of the rat. A lower activity is found in rat liver.

The T-IDH shows large variations between the organs and thus is surpassed by the activity of the D-IDH in the mitochondria of brain and locusta flight muscle. In the mitochondria of skeletal muscle the two dehydrogenases display about equal activities. On the other hand the T-IDH is found several fold higher than the DPN specific enzyme in heart and kidney mitochondria.

Organ	DPN specific Isocitrate Dehydrogenase	TPN specific Dehydrogenase
	$10^3 \frac{\mu moles}{hr \cdot gr\ protein}$ *	
Locusta, flight muscle	20	3.5
Rat, skeletal muscle	14	13
heart	8.5	37
kidney	4.7	20
brain	4.1	0.9
liver	2.6	5.5
Pigeon, heart	10	21

* μmoles pyridine nucleotide reduced per hour per gram mitochondrial biuret protein.

Table 1. Activities of DPN- and TPN specific isocitrate dehydrogenase (D-IDH and T-IDH) in mitochondria of different organs.

Discussion:

The previous failures to demonstrate convincingly the importance of the DPN specific isocitrate dehydrogenase for isocitrate oxidation in mitochondria are in part related to an insufficient knowledge (1) of the instability of D-IDH during the extraction from the mitochondria and (2) of the specific activation of this enzyme by ADP (Chen and Plaut, 1962, Goebell and Klingenberg, 1963 a). Only after the suitable extraction and assay conditions which include the consideration of the substrate and coenzyme requirement as well as pH have been worked out, the quantitative estimation of the D-IDH content in different mitochondria was feasable.

Further it is considered that enzyme activities in mitochondrial extracts, obtained after high dilution of interfering coenzymes, are a much more appropriate, since more quantitative indication of the catalyzed pathway, than the oxygen uptake or dyestuff reduction by mitochondria, which have been pretreated under difficultly reproducable conditions. It may be mentioned that with the new knowledge of the properties of the D-IDH the effectiveness of the DPN

linked pathway in isocitrate oxidation could be demonstrated also in intact mitochondria (Goebell and Klingenberg, 1963 b).

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