CONTROL OF DPN-SPECIFIC ISOCITRIC DEHYDROGENASE ACTIVITY BY PRECURSOR ACTIVATION AND END PRODUCT INHIBITION*

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The purpose of this communication is to describe some salient features of inhibition and activation *in vitro* of DPN-specific isocitric dehydrogenase (IDH) of *Neurospora crassa* which may have significance in the metabolic regulation of the tricarboxylic acid cycle. It is the general consensus of opinion that, out of the two IDH found in most organisms (one DPN- and another TPN-specific) the DPN-specific IDH is primarily responsible for the oxidation of *d*-isocitrate (3, 6).

The DPN-specific IDH was purified from *N. crassa* extracts approximately 100-fold by pH and alcohol precipitation. The preparation lacked most of the interfering enzymes, like TPN-specific IDH, α-ketoglutarate dehydrogenase, glutamic dehydrogenase and aconitase. DPN-

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specific IDH was assayed by absorbancy change at 340 mμ in a mixture containing, 4.9 μmoles isocitrate, 1.0 μmole adenosine-5'-phosphate, 120 μmoles Tris, 9.0 μmoles $MgSO_4$, 0.75 μmoles DPN, water and enzyme to make 3.0 ml. Final pH after mixing was 7.6. Cuvettes of 10 mm light-path were used.

The enzyme does not obey Michaelis-Menten kinetics at its pH optimum of 7.6. Plots of reciprocal of substrate concentration against reciprocal of initial velocity yield a curve which can only be approximated by a parabola (Fig. 1). Such curves seem to be characteristic of regulatory proteins, like aspartate transcarbamylase (4) and threonine deaminase (2, 10). When the reciprocal of the square of substrate concentration is plotted against the reciprocal of velocity, a linear curve is obtained which suggests that the enzyme has two substrate attachment sites, one 'active' and another 'activating'.

The enzyme is inhibited by α-ketoglutarate (end-product) and activated by citrate (precursor). Inhibition or activation is noticed only at unsaturating concentration of the substrate (Table I), suggesting competitive nature of the effect. However, α-ketoglutarate and citrate do not compete for the 'active' site, as is evidenced by the fact that at pH 6.5, when the enzyme is still active, no activation or inhibition occurs at all concentrations of isocitrate tested. It is significant that at this pH value, the enzyme obeys classical Michaelis-Menten kinetics. The 'activating' site

Table I

Inhibition and activation of isocitric dehydrogenase

The assay mixture was the same as described in the text, except that the concentration of d-isocitrate was varied as indicated. The compounds tested were added simultaneously with the substrate. A 118-fold purified enzyme stored for 7 days at -20° was used. Initial velocities only were measured. V' = O.D. change/min in presence of the compound tested; V = O.D. change/min with substrate only.

additions (M)	velocity 3.8×10^{-4} M substrate (unsaturating)	V'/V	velocity 1.3×10^{-3} M substrate (saturating)	V'/V
none	.027	-	.145	-
3.3×10^{-5} (cit)	.060	2.1	.143	1
3.3×10^{-4} (cit)	.125	4.3	.145	1
2×10^{-4} (α-KG)	.026	1.0	.146	1
6.6×10^{-3} (α-KG)	.007	0.27	.145	1
1.3×10^{-2} (α-KG)	.000	0.0	.143	1

cit = citrate; α-KG = α-ketoglutarate

of the enzyme apparently becomes 'non-functional' at this pH, and if we assume that citrate and α-ketoglutarate both compete only at the 'activating' site, it would explain the absence of inhibition or activation. A detailed kinetic study will have to be done before this question is settled.

It will be noted from table I that rather high concentrations of α-ketoglutarate are required to inhibit enzyme activity, and the physiological significance of this inhibition is uncertain, but activation by citrate occurs at low, physiologically significant concentrations. We propose that this phenomenon, which can be called 'precursor activation' may have some importance in the regulation of

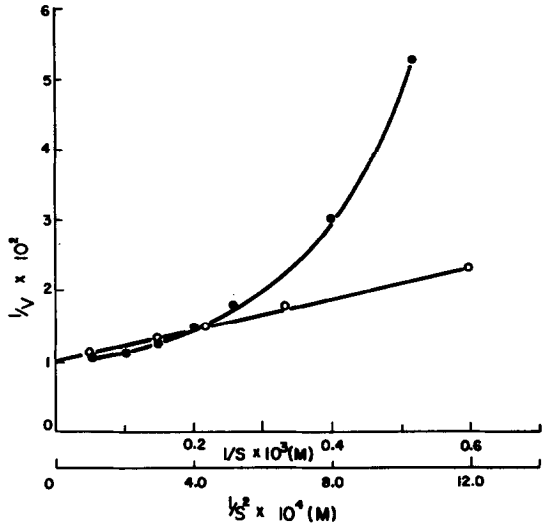

Fig. 1. Reciprocal plots of velocity against substrate (S) and S^2. ●—● , 1/V against 1/S; o—o , 1/V against $1/S^2$.

cell metabolism. It was pointed out by Krebs (7) long ago that the overall activity of a metabolic sequence which terminates in an irreversible step is governed by the activity of the enzyme catalyzing that step. However, 'precursor activation' must be distinguished from cases of non-specific activation of many enzymes by cations and anions. Criteria for this distinction are, (1) the activating compound should be a precursor, (2) the effect should be specific, (3) the activating effect must be brought about by the precursor at low, physiological concentrations, and, finally, in order to be an effective control mechanism, (4) the activated step should, for all practical purposes, be irreversible. All these criteria are met in the case of IDH. We have tested a large number of mono-, di-, and trivalent anions and cations (including cis- and trans-

aconitate, acetate, phosphate and succinate) and find citric acid completely specific for the activation of DPN-specific IDH.

Our contention that 'precursor activation' is perhaps an important control mechanism is also supported by the fact that more than one case of this kind is known. The activation of glycogen synthetase by gluocse-6-phosphate (1, 8, 9, 10) is strikingly similar to our case, and the activation of chitin synthetase by N-acetylglucosamine (5) may turn out on reinvestigation to be another example of this phenomenon.

It may have some significance that precursor activation is known in reaction sequences, the metabolic intermediates of which do not serve as building blocks for cell growth. Herein may lie the physiological importance of this control mechanism. If citrate, for some reason, is produced by the cells in excess, it would only be excreted out, but by having a precursor activation mechanism, it is ensured that citrate will be rapidly metabolized and put to use by the cells, i.e., generate energy.

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