Nicotinamide Adenine Dinucleotide Phosphate Linked Isocitrate Dehydrogenase. Catalytic Activation by the Reduced Coenzyme Product of the Reaction†

Marie-France Carlier and Dominique Pantaloni

ABSTRACT: The oxidative decarboxylation of D-isocitrate catalyzed by NADP-linked isocitrate dehydrogenase is activated by NADPH, the product of the reaction. We analyzed the autocatalytic behavior exhibited by the enzyme during the steady-state kinetics. NADP acts as a competitive inhibitor toward NADPH in the catalytic activation. In a large concentration range of the reduced and oxidized coenzymes, the activity of the enzyme is proportional to the ratio (NADPH)/(NADP). The results are compared with the results of experiments done with other NADP-linked decarboxylating dehydrogenases. Two different models are presented in order to explain the mechanism of action of isocitrate dehydrogenase, according to our data.

In order to understand the mode of regulation of an NADP-dependent decarboxylating dehydrogenase, we have studied the catalytic mechanism of cytoplasmic isocitrate dehydrogenase purified from beef liver. Several different isocitrate dehydrogenases are known to exist in mammalian tissues: an NAD^+-dependent, mitochondrial isocitrate dehydrogenase having allosteric properties (Plaut et al., 1974), a monomer $NADP^+$-dependent mitochondrial enzyme (Colman et al., 1970), whose activity, regulated by metal cations, has been studied in detail (Villafranca and Colman, 1971; Colman and Villafranca, 1971; Colman, 1971), and a little-studied dimeric cytoplasmic isozyme. Evidence from our previous work shows that this latter enzyme can utilize two substrates, either tribasic D-isocitrate in free form or D-isocitrate complexed with a divalent metal (metal-isocitrate$^-$), and that the enzyme exhibits different affinity and turnover number for each of the two substrates (Carlier and Pantaloni, 1976). We have further shown that the enzyme exhibits autocatalytic activation when in a reaction medium deprived of divalent metal cations (Carlier and Pantaloni, 1973). While for the majority of the dehydrogenases the steady state is established within a fraction of a second (Iwatsubo and Pantaloni, 1967; Shore and Gutfreund, 1970), in the case of this enzyme the establishment of the steady state is preceded by a phase of acceleration of the catalysis which can last several minutes.

We report here a detailed study of this autoactivation phenomenon. The results obtained indicate that the product NADPH plays the role of activator during the course of catalysis. The results are interpreted by examining different possible functions of the coenzyme during the catalytic cycle of dehydrogenation–decarboxylation. The possibility of extending the model proposed to explain catalysis by other $NADP^+$-dependent decarboxylating dehydrogenases is also discussed. Finally, we discuss the physiological significance of the phenomenon.

Material and Methods

The salts used in the buffers were Merck analytical grade, DL-isocitrate, $NADP^+$, and NADPH were purchased from Sigma, and crystalline diaphorase of *Klostridium kluyverii* was from Worthington.

All solutions used, including enzyme solutions, were freed of any trace metal cations by treatment with Chelex-100 resin (Bio-Rad) which had been previously equilibrated with 0.1 M phosphate buffer (pH 7.6), the buffer used throughout the work.

$NADP^+$-dependent cytoplasmic isocitrate dehydrogenase was purified from beef liver by the method described previously (Carlier and Pantaloni, 1973).

Kinetic experiments were carried out at 20 °C in 0.1 M phosphate buffer (pH 7.6). The appearance of NADPH was followed at 340 nm on a Beckman Acta V spectrophotometer, and recorded on the expanded scale (optical density = 0–0.1).

Results

(1) *Effect of Divalent Metal Ions on the Activation Phenomenon.* In the presence of very low concentrations of Mg^{2+} or Mn^{2+} (<1 μM), the isocitrate dehydrogenase shows a very low initial rate of oxidative decarboxylation of isocitrate. However, this rate increases in the course of the reaction, before reaching a steady state for which the rate remains constant for several minutes. This phenomenon of catalytic activation appears to be qualitatively dependent on the Mg^{2+} (or Mn^{2+}) concentration of the reaction mixture: the higher the Mg^{2+} concentration, the higher the initial velocity and the faster the steady state is reached (Figure 1).

We have previously shown that the enzyme can utilize, with very different affinities, two substrates, free isocitrate (I^{3-}) and isocitrate complexed with a metal (MI^-) (Carlier and Pantaloni, 1976). The fact that the catalytic activation [characterized here, as a first approximation, by the ratio v(steady state)/v(initial)] is maximal in the absence of the metal and does not appear when the enzyme is saturated with MI^- would seem to indicate that the activation phenomenon is related to some property of the enzyme in relation to the substrate isocitrate^{3-}. Thus, all subsequent experiments were carried out on solutions made very poor in metal cations by passage through a Chelex-100 resin, and to which no metal ion was added.

(2) *Preliminary Experiments.* In experiments for which reagent concentrations were fixed, the enzyme was incubat-

† From the Laboratoire d'Enzymologie, C.N.R.S., 91190-Gif-sur-Yvette, France. *Received December 28, 1973.*

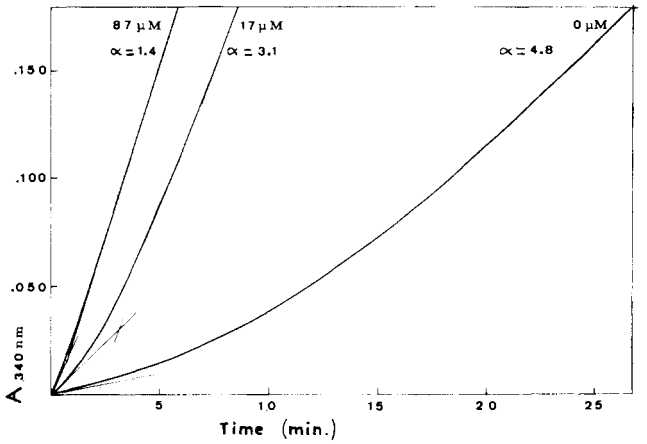


FIGURE 1: Role of Mg^{2+} in the catalytic activation. The experiments represented here were carried out in 0.2 M Tris buffer (pH 7.8). The cuvette contained 8.66 mM DL-isocitrate, 0.116 mM $NADP^+$, and Mg^{2+} added at the concentrations indicated. The reaction was begun by addition of the enzyme (final concentration, 9 nM). The ratio $\alpha = v(\text{steady state})/v(\text{initial})$ was measured for each experiment.

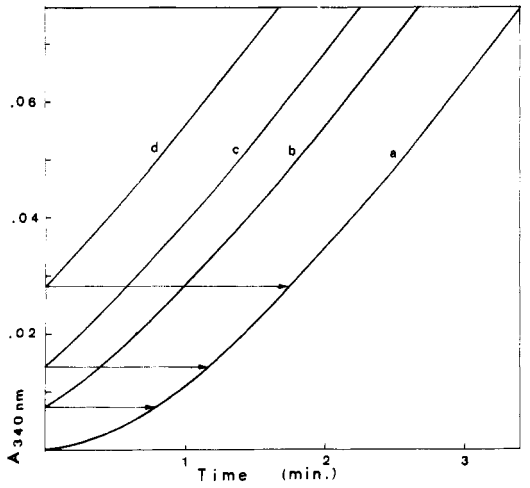


FIGURE 2: Effect of the presence of NADPH in the reaction medium at time $t = 0$ of the reaction. NADPH was added at the following concentrations: $C_a = 0$; $C_b = 1.2$ μM; $C_c = 2.4$ μM; $C_d = 4.5$ μM; [DL-isocitrate] = 8.3 mM; [$NADP^+$] = 55 μM. The reaction was started by addition of the enzyme. b, c, and d kinetics were superimposable on the a plot by following the indicated translation along the time axis.

ed, for a time period longer than that needed to reach the steady state, in the test cuvette containing either buffer alone, buffer plus isocitrate, or buffer plus $NADP^+$. The remaining reagents were added at zero reaction time. We observed no difference between the kinetic curves thus obtained and the reference curve, for which the enzyme was added to the reaction mixture at time zero.

(3) *Effect of Enzyme Concentration on the Activation Phenomenon.* Experiments were done in which only the concentration of enzyme in the test cuvette was varied, all other reagent concentrations being held constant. The recordings of the kinetics were obtained with the recording paper advancing at a speed proportional to the enzyme concentration, [E], thus permitting us to obtain an abscissa proportional to $t/[E]$. The resulting curves represent $A_{340nm} = f(t/[E])$ and are superimposable, indicating that during both the activation and steady-state phases the enzyme rate is at any time t proportional to the enzyme concentration. Thus, it would seem that the rate value is related to the value of the absorbance and thus to the concentration of one reaction product.

(4) *Effect of Product NADPH Concentration on the Course of the Reaction.* In order to study the effect of the product NADPH concentration, varying concentrations (C_i) of NADPH were added to the reaction mixture and the enzyme was added to start the reaction. As seen in Figure 2, the i curves obtained were compared to the kinetic reference ($C_i = 0$). The respective experimental curves (C_i) are superimposable on the reference curve if they are translated along the abscissa by an interval equal to the time t_i at the end of which NADPH has accumulated at the concentration C_i appearing on the reference curve. This translation is indicated by the arrows in Figure 2. When C_i is relatively high from the start of the reaction, the steady state is reached immediately. Similar addition of varying concentrations of another reaction product, 2-oxoglutarate, does not affect the kinetics.

The variation in the reaction rate during the catalytic activation phase was analyzed as a function of the product [NADPH](t). As is seen in Figure 3, the resulting plot of the double reciprocal relation:

$$\frac{[E]}{\frac{dA}{dt}(t) - \frac{dA}{dt}(0)} = f\left(\frac{1}{[NADPH](t)}\right)$$

was linear, indicating that the catalytic activation observed was the kinetic consequence of the saturation by NADPH of an active enzyme complex. To this phenomenon can be associated an "activation constant" which will be temporarily designated as:

$$K_{act} = \frac{[E^*][NADPH]}{[E^*{\cdot}NADPH]}$$

where E* represents an active enzyme complex, the nature of which will be discussed below.

The activation effect of NADPH cannot be interpreted as a slow conformation change of the enzyme complex following fixation of the reduced coenzyme, for, when NADPH is added in the reaction mixture at concentration C_i at time $t = 0$, one immediately obtains the same reaction rate as would eventually be attained at time t_i by allowing the enzyme to produce an equal concentration (C_i) of NADPH.

(5) *Effect of $NADP^+$ Concentration on Catalytic Activation.* The kinetics of catalytic activation were measured in the presence of increasing and saturating concentrations of $NADP^+$. The resulting plots are not superimposable; the same steady state is obtained, but only after a time interval which increases with increasing $NADP^+$ concentration (Figure 4A). A double reciprocal plot (see section 4 above) of the activation phase (Figure 4B) indicates competitive inhibition by $NADP^+$ of NADPH fixation, of the type: $K_{act} = K_{act}^0 (1 + [NADP^+]/K_I)$. The value of the apparent constant K_{act} increases linearly with increasing $NADP^+$ concentration (Figure 4C). Therefore, by extrapolation to zero $NADP^+$ concentration, we were able to determine an intrinsic activation constant for NADPH, $K_{act}^0 = 0.25$ μM as well as an inhibition constant for $NADP^+$ for the activation phenomenon, $K_I = 7$ μM.

The competition between $NADP^+$ and NADPH for the "activator site" of the enzyme indicates that throughout the catalytic activation phase, the measured velocity is dependent solely on the value of the ratio [$NADP^+$]/[NADPH]. Indeed, when the kinetics are measured at two different concentrations, $[NADP^+]_1$ and $[NADP^+]_2$, the resulting

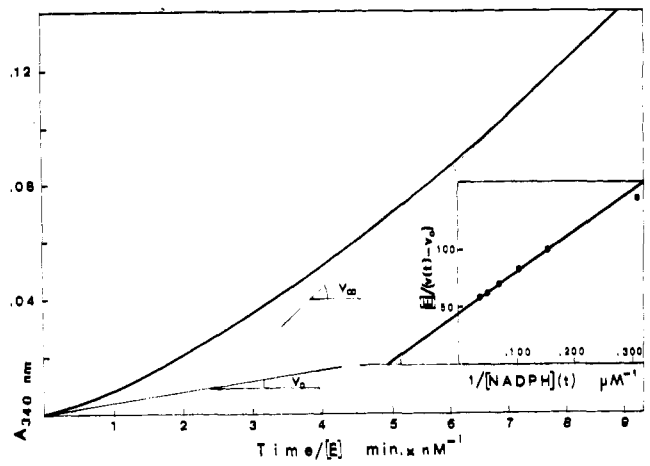


FIGURE 3: Evidence for activation by product NADPH. Recordings of the kinetics of the reaction performed in 0.1 M phosphate buffer (pH 7.55), at different enzyme concentrations, after normalization of the time scale with respect to the enzyme concentration: [DL-isocitrate] = 3.9 mM; [$NADP^+$] = 0.202 mM; the reaction was started by addition of 5, 10, 20, and 25 nM enzyme. All the kinetics were superimposable; inset: double reciprocal plots of the same kinetics.

recorded kinetics show the same reaction rate for each pair of homologous points, having respective ordinates $[NADPH]_1$ and $[NADPH]_2$, of the sort:

$$\frac{[NADPH]_1}{[NADPH]_2} = \frac{[NADP^+]_1}{[NADP^+]_2}$$

The two curves are superimposable if the time and absorbance scales are adjusted by the ratio $[NADP^+]_1/[NADP^+]_2$.

(6) *Inhibition of the Enzyme by Excess Product NADPH.* The enzyme was found to be inhibited in the steady state when NADPH was present in concentrations much higher than those used to produce the activation phenomenon. The classic inhibition is competitive with respect to NADP. In Table I are listed the intrinsic values of the dissociation constants of $NADP^+$ and NADPH implicated in the activation phenomenon and in steady-state catalysis.

(7) *Minimum Turnover for Isocitrate Dehydrogenase in the Absence of NADPH; Coupling of the Enzyme to a Diaphorase.* The phenomenon of catalytic activation by NADPH is possible only if the system shows some activity at time zero. There are two explanations for such an activity: either the enzyme is totally inactive in the absence of NADPH, and it is the reduced coenzyme present in trace amounts in the reaction medium at time zero which permits the reaction to begin at a rate dependent on the NADPH concentration, or the enzyme possesses a minimal catalytic activity in the absence of NADPH. This minimum turnover, k_0, leads to the accumulation of product NADPH, which in turn brings about catalytic activation and the establishment of the maximum turnover, k_{max}, of the fully active (steady state) enzyme. According to this hypothesis, if the NADPH produced with the turnover k_0 were to be eliminated from the system or converted rapidly to $NADP^+$ by a secondary enzyme system functioning (with the same initial velocity, v_0) in the direction NADPH → $NADP^+$, no NADPH would accumulate in the medium to activate the isocitrate dehydrogenase, which would never reach the steady-state k_{max}.

To test this hypothesis, we chose as a secondary enzyme system diaphorase, an FMN enzyme which functions as an NADPH oxidase. Oxygen bubbled into the reaction medi-

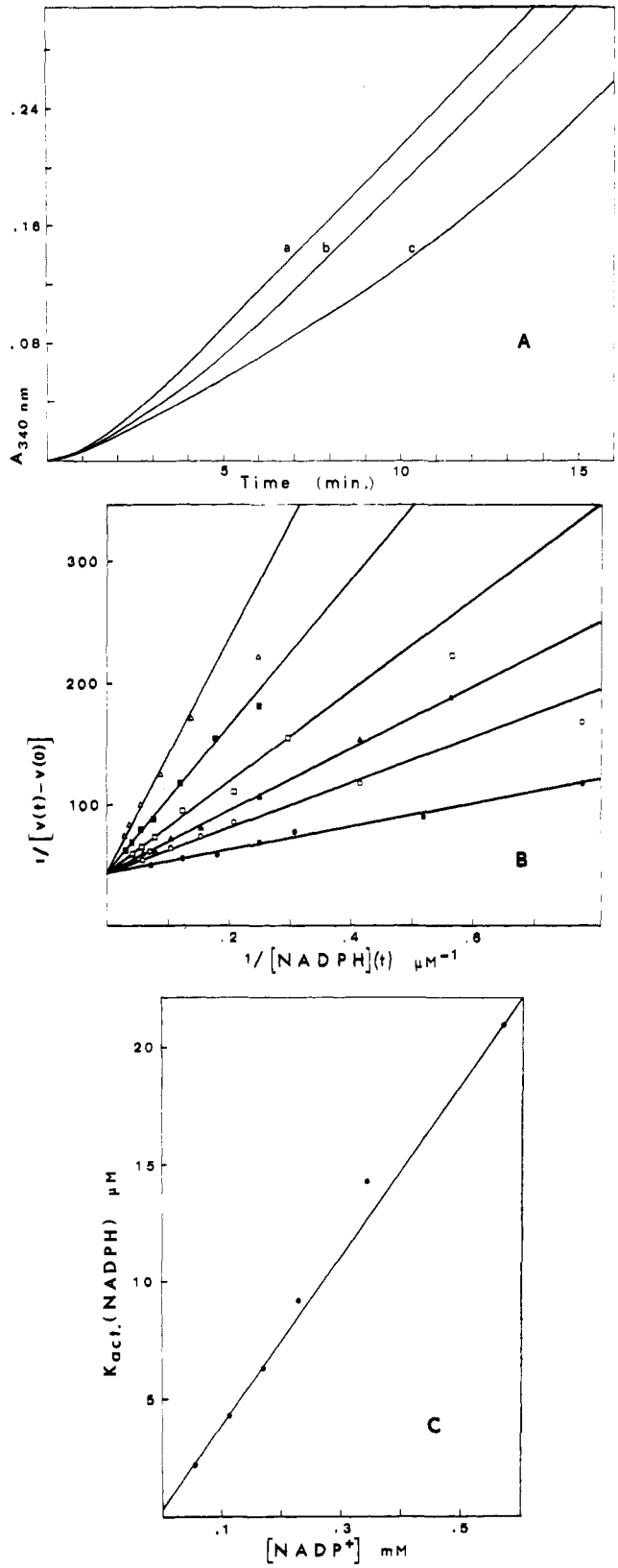


FIGURE 4: Competition of $NADP^+$ with respect to NADPH for the activation site. (A) Recorded kinetics with variable [$NADP^+$]: (a) 57 μM; (b) 229 μM; (c) 571 μM. The reaction was performed in 0.1 M phosphate buffer (pH 7.55), containing 4.46 mM DL-isocitrate and 12 nM enzyme. The c kinetics can be superimposed on the b plot if the coordinates of each point of c are divided by 571/229 = 2.5. (B) Double reciprocal plots of the same experiments. $NADP^+$ concentrations are as follows: (●) 57 μM; (○) 114 μM; (▲) 171 μM; (□) 229 μM; (■) 343 μM; (△) 571 μM. (C) Variation of K_{act}(NADPH) with [$NADP^+$], from the same experiments. The values $K_I'(NADP^+)$ = 7 μM and K_{act}^0(NADPH) = 0.25 μM were determined.

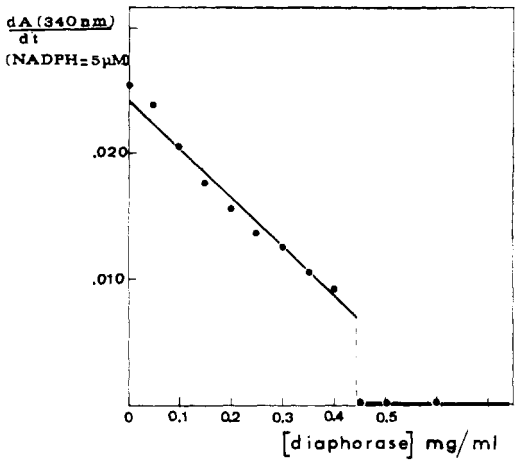


FIGURE 5: Evidence for a minimum activity of isocitrate dehydrogenase in the absence of NADPH. The reaction was performed in a reaction medium deprived of metal cations and containing 0.1 M phosphate buffer (pH 7.4), 8.1 mM DL-isocitrate, 0.24 mM $NADP^+$, and diaphorase at variable concentrations. The reaction was started by addition of 12 nM ICDH. The slope dA_{340}/dt was measured on the recorded graphs of the reaction at the time when 5 μM NADPH was produced in the medium ($\Delta A_{340} = 0.030$).

Table I: Values of the Intrinsic Dissociation Constants for $NADP^+$ and NADPH at the Catalytic and Activation Sites.

Ligand	Catalytic Site	Activator Site
$NADP^+$	$K_m = 7\ \mu M$	$K_I' = 8\ \mu M$
NADPH	$K_I = 35\ \mu M$	$K_{act} = 0.25\ \mu M$

um was used as electron acceptor. Diaphorase was chosen for the following reasons. (1) In the experimental conditions used, the affinity of diaphorase for NADPH is $\geq 2 \times 10^7$ M^{-1} and the oxidation-reduction equilibrium $NADP^+$-NADPH is greatly displaced in favor of $NADP^+$ formation, so that even in the presence of high concentrations of $NADP^+$, all NADPH present is oxidized at a constant rate, no matter what its concentration is, until a concentration $\leq 0.5\ \mu M$ (limit of detectability) is reached. (2) The activity of diaphorase is proportional to its concentration. (3) The presence of diaphorase in a reaction medium deprived of oxygen by bubbling through nitrogen does not affect isocitrate dehydrogenase activity.

In a first experiment, buffer, $NADP^+$, isocitrate dehydrogenase, and a known quantity of diaphorase were incubated in the measuring cuvette for a time sufficient to convert about 10 μM of contaminating NADPH to $NADP^+$. The reaction was then started by addition of isocitrate. We observed the same kinetics of NADPH appearance as were observed when the diaphorase was added at zero reaction time, all other conditions being held the same. Thus, the initial rate of the isocitrate dehydrogenase seems to result from an intrinsic activity of the enzyme in the absence of NADPH.

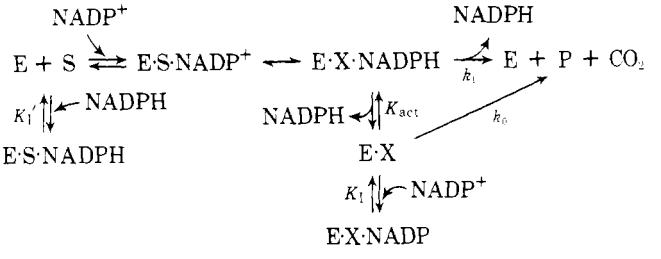
We then determined the value of the turnover k_0 of the enzyme in the absence of NADPH. The reaction was started by the addition at zero time of a fixed quantity of isocitrate dehydrogenase to a cuvette saturated with oxygen and containing the isocitrate dehydrogenase reaction mixture plus increasing concentrations of diaphorase. The rate of appearance of NADPH, either at the steady state or at the instant when a given concentration of NADPH had accumulated in the medium, was measured as a function of the diaphorase concentration. As can be seen in Figure 5, this rate decreased linearly with increasing diaphorase concentration until a point of discontinuity was reached at which NADPH no longer appeared. It can therefore be concluded that at this point the reaction rate for the conversion of NADPH to $NADP^+$ by diaphorase was equal to the rate v_0 of isocitrate dehydrogenase in the absence of NADPH. Thus, the minimum turnover k_0 of isocitrate dehydrogenase can be read from the standard curve for diaphorase, giving a value of $k_0 = 2\ s^{-1}$.

Discussion

Development of a Model. In order to interpret these results a model should include the following characteristics: (a) an enzyme having a minimum turnover k_0 and a maximum turnover k_1; (b) NADPH complexing to an active enzyme complex and increasing the enzyme turnover from k_0 to k_1; (c) an enzyme with only one NADPH binding site per subunit (unpublished results); (d) $NADP^+$ competing with NADPH for the "activator site" but not itself activating the enzyme; and (e) NADPH inhibiting the enzyme at the catalytic site in competition with $NADP^+$. Thus, the distinction between different possible models lies in the supposed nature of the "activator" complex, E*·NADPH.

The first model would explain the activation by the existence of a very rapid association-dissociation equilibrium of the NADPH in the intermediate enzyme-oxalosuccinate-NADPH complex. The binary complex enzyme-oxalosuccinate would be decarboxylated with a turnover k_0 inferior to the turnover k_1 of the tertiary complex, enzyme-oxalosuccinate-NADPH. The model can be represented by Scheme I, where S = D-isocitrate and X = oxalosuccinate. The acceleration of the catalysis and the establishment of the steady state are the kinetic representations of the displacement of the equilibrium EX + NADPH $\rightleftarrows$ E·X·NADPH toward the right which occurs upon accumulation of reduced coenzyme in the medium. This displacement would imply that the rate of NADPH dissociation from the complex is high compared to the decarboxylation rates k_0 and k_1 which are kinetically limiting.

Scheme I



This model accounts for the double competition between $NADP^+$ and NADPH, the first one which is trivial, at the level of the formation of the Michaelis complex E-S-$NADP^+$ and the other at the level of the intermediate E·X·NADPH complex.

The following equation:

$$v = [EX]\left(k_0 + k_1 \frac{[NADPH]}{k_{act}\left(1 + \frac{[NADP^+]}{K_I}\right)}\right)$$

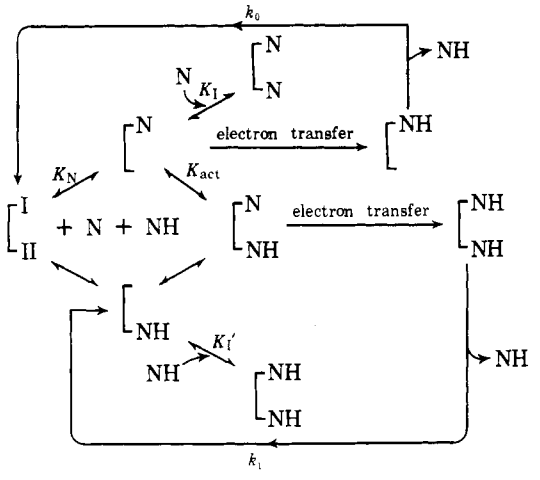
simultaneously accounts for the kinetic acceleration by

NADPH fixation and for the modulation of this activation by $NADP^+$. The alternative fixation of either $NADP^+$ or NADPH on a unique site explains both the catalysis and the activation processes.

The model does not take into account the quaternary structure of the enzyme, which is known to be a dimer (Carlier and Pantaloni, 1973). Results of experiments using commercially prepared pig heart mitochondrial isozyme, a monomeric enzyme (Colman et al., 1970), showed the same phenomenon of activation by product NADPH. These results favor a model, which implies the existence of only one site of coenzyme fixation per molecule of enzyme.

The second model proposed is of the "flip-flop" type (Lazdunski et al., 1971). Cytoplasmic isocitrate dehydrogenase is known to be a dimer which can bind two molecules of coenzyme per dimer (unpublished results). The two binding sites, indistinguishable by fluorescence titration at equilibrium, are shown to differ by the rate of fixation of NADPH to the enzyme in the presence of isocitrate (unpublished results). These observations support a hypothesis according to which the asymmetry of the enzyme would generate activation by the product, i.e., the enzyme would be in its activated state when one of the two sites (site I) is occupied by a molecule of $NADP^+$ and the other (site II), by a molecule of NADPH. Scheme II is proposed.

Scheme II



The catalytic activation is explained by the fixation of NADPH on site II of the coenzyme. The above model accounts for the competition of $NADP^+$ with NADPH at site II (activator) and that of NADPH with $NADP^+$ at site I (catalytic). Sites I and II permute at each cycle of the enzyme. Neither the substrate, isocitrate, nor the products are included in the scheme, since the experiments reported here did not give precise enough data relative to their fixation and dissociation.

The rate equation for this model is:

$$v = k_0 \frac{[E][NADP^+]}{K_{NADP^+}\left(1 + \frac{[NADPH]}{K_I'}\right)} \times \left(1 + \frac{k_1}{k_0} \frac{[NADPH]}{K_{act}\left(1 + \frac{[NADP^+]}{K_I}\right)}\right)$$

which, when $[NADP^+] \gg K_{NADP^+}$ and $[NADPH] \ll K_I'$, can be written:

$$\frac{v}{[E]} = k_0 + k_1 \left(\frac{[NADPH]\, K_I}{[NADP^+] K_{act}}\right)$$

showing that in the concentration range defined above, the enzyme activity is simply a linear function of the ratio $[NADPH]/[NADP^+]$.

It should be noted that the two models proposed are strictly identical mathematically. A functional asymmetry of the enzyme can be proved or disproved only by measuring the kinetics of coenzyme fixation under different conditions in the presence of different ligands. This work is now in progress in our laboratory.

Our results show that in the absence of added divalent metal cations isocitrate dehydrogenase activity undergoes positive regulation by product NADPH and that $NADP^+$ reverses this effect. It follows that, over a certain range of concentration of the reduced and oxidized forms of the coenzyme, there exists a phenomenon which is the inverse of the classical scheme of feedback inhibition by the product. The enzyme activity is an increasing function of the ratio $[NADPH]_{free}/[NADP^+]_{free}$. Thus, the problem which remains is to understand how the "balance" between $NADP^+$ and NADPH is brought about at the molecular level and which enzyme complex is responsible for the activator role of NADPH.

In the case of 6-phosphogluconate dehydrogenase, it has been proved that NADPH also plays a non-redox role: (1) in the catalysis of the second step (decarboxylation) of the ongoing reaction (Rippa et al., 1973); (2) in the hydrogen exchange between ribulose 5-phosphate and water, which is the first step of the reverse (carboxylation) reaction (Lienhard and Rose, 1964). Similarly, the catalysis by isocitrate dehydrogenase of the same type of hydrogen exchange between 2-oxoglutarate and water requires the presence of NADPH (Rose, 1960), even though the redox activity of the molecule is not involved (Rippa et al., 1974). It has further been shown that $NADP^+$ stimulates the oxaloacetate decarboxylation catalyzed by the malic enzyme (Rutter and Lardy, 1958). Moreover, we have demonstrated that catalytic activation of isocitrate dehydrogenase still occurs when NADPH is replaced by $NADPH_4$, a component devoid of any oxidation–reduction capacity (Carlier et al., 1976).

All of these facts tend to suggest that in the case of several $NADP^+$-dependent decarboxylating dehydrogenases, the coenzyme functions at a step ulterior to the dehydrogenation. This property might be a general characteristic of the catalytic mechanism of this class of dehydrogenases. The resulting activation phenomenon would be apparent only under those conditions in which the rate of dissociation of NADPH from the intermediate enzyme complex is much greater than the decarboxylation rate. Several factors can modify this rate ratio. For example, it would seem that the absence of activation by NADPH in the presence of a divalent metal cation is due to one of the following reasons: (a) an increase in the rate of decarboxylation of the complex E·X·NADPH by the metal ion (such a finding would be in agreement with chemical evidence concerning catalysis of the decarboxylation of β-keto acids by divalent metal ions); (b) a decrease in the rate of NADPH liberation, i.e., the stabilization of the intermediate complex; or (c) the simultaneous occurrence of the two phenomena.

We have similarly observed that the use of phosphate buffer enhances the activation phenomenon; indeed, the

HPO_4^{2-} ion, a competitive inhibitor of the oxidized coenzyme for fixation at the catalytic site, is also a competitive inhibitor of fixation of the reduced coenzyme as activator. The apparent activation constant of NADPH increases linearly with increasing HPO_4^{2-} concentration. A more detailed study of the multiple inhibition by $NADP^+$ and HPO_4^{2-} of the activator effect of NADPH has shown that the intrinsic inhibition constant for HPO_4^{2-} has the same value, 20 mM, with respect both to the activator site (NADPH) and the catalytic site ($NADP^+$). This would seem to confirm that the activator and catalytic sites are localized in the same region of the enzyme molecule, at least with respect to fixation of the phosphate of the coenzyme molecule.

Other examples of product-activated enzymatic reactions are found in the literature, systems which, exhibiting nonlinear kinetics, can generate oscillations. The most familiar example is that of glycolysis (Hess and Boiteux, 1968). The first mediator of oscillations in glycolysis is the key enzyme phosphofructokinase, for which a mathematical model has been described (Sel'Kov, 1968). It seems probable that isocitrate dehydrogenase is capable of producing the same type of oscillations in an open system on which are imposed the appropriate conditions of $NADP^+$ entry and NADPH escape. Indeed this enzyme exhibits characteristics of two different types of dynamic behavior (two turnover extremes) which are a reflection of the nonlinear kinetics.

Finally, the possible physiological implication of these findings merits mention. The concentration ratio $[NADPH]_{total}/[NADP^+]_{total}$ in the different cellular compartments is known (Sols and Marco, 1968), the value being 3 in the cytoplasm of mammalian tissue cells. Since the majority of the $NADP^+$ dehydrogenases show a much greater affinity for NADPH than for $NADP^+$, the enzymes are generally in a state of nonequilibrium in the cytoplasm because of the inhibition by NADPH. However, the situation is reversed for isocitrate dehydrogenase with the NADPH stimulating its own production by the enzyme. Thus, it might be suggested that the isocitrate dehydrogenase plays a role in reestablishing the coenzyme equilibrium.

Acknowledgments

We are indebted to France Tejtel-Eisenhower for her assistance in the translation of the manuscript.

References

Carlier, M. F., et al. (1976), *FEBS Lett., 62,* 236.
Carlier, M. F., and Pantaloni, D. (1973), *Eur. J. Biochem. 37,* 341–354.
Carlier, M. F., and Pantaloni, D. (1976), *Biochimie* (in press).
Colman, R. F. (1971), *Ann. N.Y. Acad. Sci. 141,* 1–13.
Colman, R. F., Szeto, R. C., and Cohen, P. (1970), *Biochemistry 9,* 4945–4948.
Colman, R. F., and Villafranca, J. J. (1971), *J. Biol. Chem. 247,* 215–219.
Hess, B., and Boiteux, A. (1968), in Regulatory Functions of Biological Membranes, Amsterdam, Elsevier, pp 148–162.
Iwatsubo, M., and Pantaloni, D. (1967), *Bull. Soc. Chim. Biol. 49,* 1563–1572.
Lazdunski, M., Petitclerc, C., Chappelet, D., and Lazdunski, C. (1971), *Eur. J. Biochem. 20,* 124–139.
Lienhard, G. E., and Rose, I. A. (1964), *Biochemistry 3,* 190–195.
Plaut, G. W. E., Shramm, V. L., and Aogaichi, T. (1974), *J. Biol. Chem. 249,* 1848–1856.
Rippa, M., Signorini, M., and Dallochio, F. (1973), *J. Biol. Chem. 248,* 4920–4925.
Rippa, M., Signorini, M., and Dallochio, F. (1974), *FEBS Lett. 39,* 24–26.
Rose, Z. B. (1960), *J. Biol. Chem. 235,* 928–933.
Rutter, W. J., and Lardy, H. A. (1958), *J. Biol. Chem. 233,* 374.
Sel'Kov, E. E. (1968), *Eur. J. Biochem. 4,* 79–86.
Shore, J. D., and Gutfreund, H. (1970), *Biochemistry 9,* 4655–4659.
Sols, A., and Marco, R. (1968), *Curr. Top. Cell. Regul. 2,* 227–273.
Villafranca, J. J., and Colman, R. F. (1971), *J. Biol. Chem. 247,* 209–214.