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Comparison of the Energetics of the Uncatalyzed and Glutamate Dehydrogenase Catalyzed α-Imino Acid-α-Amino Acid Interconversion†

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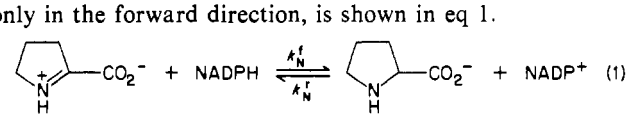
ABSTRACT: The thermodynamic and activation parameters for the reduction of Δ^1-pyrroline-2-carboxylic acid (an α-imino acid) by reduced nicotinamide adenine dinucleotide phosphate (NADPH) are compared with those for the reduction of the same imino acid by the glutamate dehydrogenase–NADPH complex. The enthalpies of activation and standard free energy changes for these two reactions are found to be virtually the same. The catalysis by the enzyme, expressed as the ratio of the reactivity of the enzyme–NADPH complex to that of NADPH itself in reducing the iminium ion, is entirely accounted for by a more favorable entropy of activation with enzyme–NADPH as the reductant. This entropic driving force is large enough to overcome the exergonic formation of the binary complex and still lead to considerable catalysis by glutamate dehydrogenase. Comparison of $\Delta S^{\ddagger}$ and ΔS° values for the reduction of the iminium ion by NADPH suggests that the solvation of the transition state resembles that of the reactants, even though the substituent effects on rate have shown that the hydride transfer from the reduced coenzyme is complete at the transition state [Srinivasan, R., Medary, R. T., Fisher, H. F., Norris, D. J., & Stewart, R. (1982) *J. Am. Chem. Soc. 104*, 807]. The ΔG° and $\Delta S^{\ddagger}/\Delta S^{\circ}$ values for the reduction by the enzyme–NADPH complex indicate that this reaction has a fairly symmetric transition state, the solvation properties of which are intermediate between those of the reactants and those of the products. Both the enzyme-catalyzed model reaction and the transient phase of glutamate reaction involve the hydride transfer step, and in both cases, the enthalpy barriers are larger than the corresponding entropy barriers.

We have previously employed the stable α-imino acid Δ^1-pyrroline-2-carboxylic acid (PCA)[1] as a model for α-iminoglutarate (Srinivasan et al., 1982). Iminoglutarate, in the enzyme-bound state, occurs as a reactive intermediate in the glutamate dehydrogenase catalyzed reductive amination of α-ketoglutarate (Brown et al., 1978; Hochreiter et al., 1972; Fisher & Viswanathan, 1984). The cyclic imino acid is reduced to DL-proline by several 1,4-dihydropyridines including reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Meister et al., 1957; Srinivasan et al., 1982). The nonenzymatic reaction, which proceeds at a measurable speed only in the forward direction, is shown in eq 1.



We have previously reported that the nonenzymatic reaction has the following features (Srinivasan et al., 1982): (1) the iminium ion is the active oxidant, (2) the rates for the reduction

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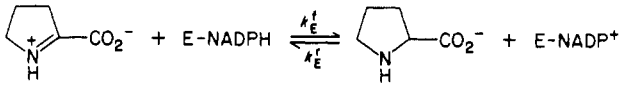
[1] Abbreviations: PCA, Δ^1-pyrroline-2-carboxylic acid; E, glutamate dehydrogenase; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; Tris, tris(hydroxymethyl)aminomethane.

of PCA by several 1-substituted 1,4-dihydronicotinamides correlate with the redox potentials of the reductants to give a unit Brønsted exponent, and (3) the reduction of the imino acid by NADH and [4,4-^{2}H]NADH has an observed kinetic isotope effect (k^{HH}/k^{DD}) of 1.5. It was noted that the reduction of PCA by various 1,4-dihydronicotinamides deuterated at C-4 produced no significant disparity between the primary kinetic deuterium isotope effect and the partition isotope effect (Srinivasan & Fisher, 1985b).

The reduction of PCA is also catalyzed by glutamate dehydrogenase[2] (where only the L isomer of the amino acid participates in the reaction), and the rates are now measurable in the forward and reverse directions (Fisher et al., 1982; Srinivasan & Fisher, 1985a). The enzyme-catalyzed reaction, representing the reversible reduction of PCA by the enzyme–NADPH complex, is shown in eq 2.



(2)

We have studied the enzyme-catalyzed reaction from the pH dependences of k_E^f and k_E^r (eq 2) (Srinivasan & Fisher, 1985a). This reaction has the following features: (1) the iminium ion and proline anion are the active substrates in the forward and reverse directions, respectively; (2) the enzyme–NADPH complex is about 500 times more reactive than NADPH itself in reducing PCA at neutral pH, and this factor increases to about 7500 at pH >10 where an unprotonated group Z, of pK = 8.6 in the enzyme–NADPH complex, further facilitates the reaction by about 15-fold. Furthermore, a strict first-order dependence with respect to the substrate was observed over a wide range of substrate concentrations in both forward and reverse directions (Srinivasan & Fisher, 1985a; Fisher et al., 1982). It is apparent, therefore, that the reason for only modest catalysis of the model reaction by glutamate dehydrogenase is the poor binding of PCA to enzyme–NADPH and of proline to enzyme–NADP$^+$; the turnover number itself is quite large. The absence of a second carboxylate group (corresponding to the γ-carboxylate group in α-ketoglutarate and glutamate) in the model compounds and their cyclic structures must be responsible for such poor binding to the enzyme–coenzyme complex.

We now report the thermodynamic and activation parameters for the reduction of the iminium ion of PCA by NADPH (eq 1) and compare them with the parameters for the reduction of the same imino acid by E–NADPH (eq 2). The latter reaction was studied under conditions where the Z in E–NADPH is in the protonated state. Since these parameters for both reactions pertain to unitless equilibrium constants and second-order rate constants, we are able to study the energetics associated with the catalysis by glutamate dehydrogenase without the ambiguity that often arises when nonenzymatic and enzymatic reactions are compared (Jencks, 1969).

Materials and Methods

Materials. L-Proline from Sigma was purified by repeated crystallization from aqueous ethanol. α-Keto-δ-aminovaleric acid was synthesized as the hydrochloride salt (Hasse & Wieland, 1960). This compound spontaneously cyclizes to PCA upon dissolution in water at pH >2 (Cabello et al., 1964; Macholan & Vencalkova, 1963). NADPH from Boehringer-Mannheim and NADP$^+$ from Sigma were used without further purification. Bovine liver L-glutamate dehydrogenase was obtained as an ammonium sulfate suspension from Boehringer-Mannheim and treated with Norit A to remove nucleotides (Cross & Fisher, 1970). The concentrations of the enzyme, NADPH, and NADP$^+$ solutions were determined spectrophotometrically (Johnson et al., 1981).

Thermal Denaturation Studies with Glutamate Dehydrogenase. Glutamate dehydrogenase (190 μM) in 0.1 M phosphate buffer at pH 7.60 was denatured at 55.9 ± 0.1 °C for 2 min. The precipitated enzyme was removed by centrifugation, and the supernatant was assayed for its activity by adding the same volume as that for the nondenatured enzyme solution to reaction mixtures. The supernatant was found to be 69 ± 1% active relative to the nondenatured enzyme solution as measured by its activity toward both the α-ketoglutarate and PCA reactions. When the enzyme was denatured for 2.5 min under the same conditions, both assay methods gave 39 ± 1% activity for this enzyme solution.

Kinetic Procedure. The rates for the nonenzymatic reaction were measured (Srinivasan et al., 1982) over a temperature range of 5–40 °C in 0.1 M Tris–0.01 M phosphate–0.05 M PCA buffer at pH(25 °C) 7.0, and they were corrected for the general-acid-catalyzed decomposition of the reduced coenzyme (Norris & Stewart, 1977; Srinivasan et al., 1982). The rates for the enzymatic reduction of PCA were measured in the same buffer at pH(25 °C) 7.0, with [E] at 6 μM and [NADPH] at 100 and 200 μM over the temperature range of 9–35 °C, while those for the enzymatic oxidation of L-proline were measured in 0.1 M Tris–0.01 M phosphate–1 M L-proline buffer at pH(25 °C) 9.2, with [E] at 23 μM and [NADP$^+$] at 1.5 and 3.0 mM over the temperature range of 5–35 °C. The rates were then extrapolated to infinite coenzyme concentration. To obtain the rate constant for the reduction of the iminium ion by NADPH and enzyme–NADPH at a given temperature, the concentration of the iminium ion was calculated from the pH of the solution and from the pK both measured at that same temperature. The rate constant for the reduction of proline anion by E–NADP$^+$ at any temperature was similarly determined. The pK values of PCA and proline at 25 °C are 6.05 (Srinivasan et al., 1982) and 10.64 (Christensen et al., 1976), respectively, and their standard enthalpies of ionization are 9.38 (Srinivasan et al., 1982) and 10.31 kcal/mol (Christensen et al., 1976), respectively.

Determination of the Equilibrium Constant. The equilibrium condition for the PCA ~ proline reaction was obtained by adding a small amount of enzyme to a solution containing the reactants and products and noting the pH at which there was no observable production or depletion of NADPH (Srinivasan & Fisher, 1985a). In computing the equilibrium constant K_N (which is k_N^f/k_N^r in eq 1) at a given temperature, we used the pH and pK values of the imino and amino acids measured at that same temperature. The equilibrium constants were determined over a range of PCA, NADPH, and NADP$^+$ concentrations and at several pH values; the concentration of

[2] The following observations strongly suggest that the enzyme catalyzing the PCA reaction is indeed glutamate dehydrogenase: (1) the ratio of reactivity of the nondenatured and thermally denatured enzyme solutions is the same when assayed by PCA and α-ketoglutarate reactions (see Materials and Methods); (2) only the B-side hydrogen of the reduced coenzyme is abstracted in these reactions (Srinivasan & Fisher, 1985b); (3) NADH and NADPH are effective for the enzymatic reductions of PCA and α-ketoglutarate (Srinivasan & Fisher, 1985b); (4) both these reactions are inhibited by oxalylglycine and L-glutamate, and the PCA reaction is also inhibited by α-ketoglutarate (Fisher et al., 1982); (5) L-proline and L-glutamate, but not their D isomers, are oxidized by NADP$^+$ enzymatically (Srinivasan & Fisher, 1985a); (6) beef heart lactate dehydrogenase, a common impurity in glutamate dehydrogenase solution, is less than 1% as effective as glutamate dehydrogenase in reducing PCA (Fisher et al., 1982).

Table I: Thermodynamic Constants for the Reduction of PCA (Imino Group Protonated) by NADPH and E-NADPH at 25 °C

reductant	equilibrium constant	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)
NADPH	280 ± 50[a]	−3.3 ± 0.1	6.4 ± 1.0[c]	33 ± 4
E-NADPH	175 ± 33[b]	−3.1 ± 0.1	5 ± 1	27 ± 4

[a] Refers to k_N^f/k_N^r in eq 1. [b] Refers to k_E^f/k_E^r in eq 2. [c] Determined calorimetrically. A value of 8.6 ± 2.0 kcal/mol was obtained from the temperature dependence of K_N.

proline was, however, always kept at ~1 M. The equilibrium constants were found to be constant over this range of variables at 280 ± 50 at 25 °C. We noted that in the temperature range of 8–33 °C, over which K_N was measured, the coenzymes and PCA were stable during the time period required for equilibrium measurements.

Heat of Reaction. The $\Delta H°$ value for the reduction of PCA by NADPH at 25 °C was determined by using an LKB 10700-2 batch microcalorimeter with thermopile output amplified by a Keithley 150B millimicrovoltmeter. A recorder provided additional amplification.

In a typical experiment, about 4 mL of enzyme–PCA solution and 2 mL of NADPH solution (both in 0.1 M phosphate buffer at pH 6.98) were introduced by syringes in the reaction cell chambers of the calorimeter. The exact volumes of the sample were determined by weighing the syringe prior to and after sample injections. The reaction cell was balanced by equal volumes of water in the reference cell chambers. After equilibration of the solutions in the chambers, the solutions were mixed; the concentration of NADPH solution at the instant of mixing was determined spectrophotometrically by using an aliquot of the solution thermostated at 25 °C in a water bath. The concentrations of PCA, NADPH, and glutamate dehydrogenase in the reaction cell were 44 mM, 610 μM, and 11 μM, respectively. The heat produced in the reaction cell was recorded for 1 h, the time required for >95% completion of the reaction. Appropriate corrections were made for the small heat of dilution of the enzyme–PCA solution. The heat of formation of the enzyme–NADPH complex and the heat of dilution of the NADPH solution were found to be negligible under the experimental conditions. The heats of ionization of dihydrogen phosphate, PCA, and proline were taken to be 0.9 (Christensen et al., 1976), 9.38, and 10.31 kcal/mol, respectively. The observed heat of the reaction was determined by cutting out the signal from the chart paper and weighing it; electrical calibrations, tested with the known heat of dilution for sucrose, were used as standards. The standard heat of reaction $\Delta H°$ for the reduction of the iminium ion of PCA by NADPH to give proline anion and $NADP^+$ was calculated from the observed heat and the $\Delta H°$ values for the ionization of $H_2PO_4^-$, PCA, and proline.

Kinetic and Thermodynamic Constants for the Haldane Equation. The kinetic constants were measured in 0.1 M Tris–0.01 M phosphate–1 M proline–0.05 M PCA buffer at pH 8.85 and at 25 °C. The Michaelis constant K_{NADPH} was also determined under the same experimental conditions. We found that K_{NADP^+} calculated from proline oxidation rates

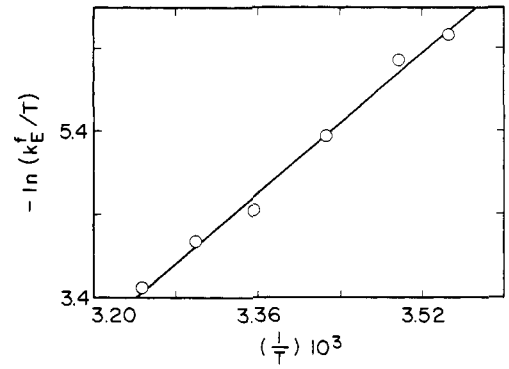


FIGURE 1: Temperature dependence of the reduction of PCA by the E–NADPH complex. [E] = 0.35 mg/mL; [PCA] = 0.05 M; [Tris] = 0.1 M; [phosphate] = 0.01 M; pH at 25 °C = 7.0. The k_E^f value is calculated at infinite concentration of NADPH (see Materials and Methods).

depends upon the range of coenzyme concentration employed (Srinivasan & Fisher, 1985a). The dissociation constant for the enzyme–NADPH complex $K_{i,NADPH}$ was obtained by calorimetric titration in 0.1 M Tris–0.01 M phosphate–1 M proline buffer at the same pH and temperature.

Results

The standard free energy change for the reduction of the iminium ion by NADPH (eq 1) was obtained from the equilibrium constant K_N; the $\Delta H°$ value for the reaction was determined calorimetrically and from the temperature dependence of K_N. The thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $\Delta S°$) for the reduction of the iminium ion by the enzyme–NADPH complex (eq 2) were calculated from k_E^f and k_E^r and from their temperature coefficients. These parameters for the reactions involving NADPH and enzyme–NADPH as reductants are collected in Table I.

The activation parameters ($\Delta G^‡$, $\Delta H^‡$, and $\Delta S^‡$) for the reduction of the iminium ion by NADPH and enzyme–NADPH in both the forward and reverse directions are given in Table II. The temperature dependences of k_N^f, k_E^f, and k_E^r reported in Table II were determined from Eyring plots, a typical plot being shown in Figure 1. Since the oxidation of the proline anion by $NADP^+$ was too slow to measure, we have calculated $\Delta H^‡$ and $\Delta S^‡$ for this process from the thermodynamic constants for the equilibrium in eq 1 and from the activation parameters for the reduction of the iminium ion by NADPH.

We construct a free energy profile for the enzyme-catalyzed reaction by employing the Haldane equation (eq 3), where K_{Pro} represents the acid ionization constant of proline (pK_{Pro} = 10.64) and K_{PCA} that of PCA (pK_{PCA} = 6.05). The values

$$\frac{k^f_{obsd}K_{i,NADP^+}}{k^r_{obsd}K_{i,NADPH}} = \frac{K_N([H^+]/K_{Pro} + 1)}{K_{PCA}/[H^+] + 1} \quad (3)$$

of k^f_{obsd} (representing the rate of reduction of 1 M total PCA by 1 M E–NADPH) and k^r_{obsd} (representing the rate of oxidation of 1 M total proline by 1 M E–$NADP^+$) are 0.024 M^{-1} s^{-1} and 2.4 × 10^{-4} M^{-1} s^{-1}, respectively. $K_{i,NADPH}$ was deter-

Table II: Rate Constants and Activation Parameters for the Reversible Reduction of PCA (Imino Group Protonated) by NADPH and Enzyme–NADPH at 25 °C

reaction	rate constant (M^{-1} s^{-1})	$\Delta G^‡$ (kcal/mol)	$\Delta H^‡$ (kcal/mol)	$\Delta S^‡$ (eu)
nonenzymatic (eq 1)	k_N^f = (7.0 ± 0.7) × 10^{-3}	20.4 ± 0.1	19 ± 1	−5 ± 3
	k_N^r = (2.5 ± 0.6) × 10^{-5}	23.7 ± 0.1	13 ± 1	−38 ± 3
enzymatic (eq 2)	k_E^f = 3.5 ± 0.4	16.7 ± 0.1	21 ± 1	14 ± 3
	k_E^r = 0.020 ± 0.003	19.7 ± 0.1	16 ± 1	−13 ± 3

mined to be 72 ± 20 μM from the kinetics of the PCA reaction and from calorimetric titration. The dissociation constant of E–NADP$^+$, $K_{i,NADP^+}$, is estimated to be 20 μM from eq 3.

Discussion

Mechanism of Catalysis of the Model Reaction by Glutamate Dehydrogenase from the Energetics Viewpoint. The nonenzymatic reduction of PCA by dihydronicotinamides proceeds faster when electron-releasing substituents are introduced at the N-1 position in the dihydronicotinamides (Srinivasan et al., 1982); the same substituents also make the reaction more exergonic. Since the rates of reduction of PCA by 1-substituted dihydronicotinamides correlate with the redox potentials of the latter to give a unit Brønsted coefficient (Srinivasan et al., 1982), the increase in the stability of products over reactants—the thermodynamic drive—accompanying the introduction of electron-releasing substituents is completely expressed in the rate constants. However, we have found that the enzyme–NADPH complex is about 500 times more reactive than NADPH itself, yet the $\Delta G°$ values for the reactions in eq 1 and 2 are quite similar (Table I). It is apparent, therefore, that the enhanced reactivity of E–NADPH over NADPH itself in reducing PCA is not due to an enhancement in the thermodynamic drive for the reaction with the former reductant. Comparing now the activation parameters (Table II) for the reduction of the iminium ion by NADPH and E–NADPH, we note that their enthalpies of activation are similar. The entropy of activation, however, aids the oxidation of E–NADPH to such an extent that the increased reactivity of this complex over NADPH is attributable solely to a more favorable entropy of activation with the former reductant. *Thus, the enzyme achieves catalysis entirely by reducing the entropy barrier;*[3] *the enthalpy barrier and the thermodynamic drive are virtually unaffected.*

Entropy of Activation. The negative $\Delta S^‡$ values for the oxidation of the proline anion by NADP$^+$ and by enzyme–NADP$^+$ cannot be ascribed simply to the loss of rotational and translational entropies accompanying the combination of two molecules to form a monomolecular transition state (Page & Jencks, 1971) since, by the same argument, these entropies must be lost in the forward reaction also. However, $\Delta S^‡$ is actually positive and near zero for the reduction of PCA by E–NADPH and NADPH, respectively. Furthermore, the equilibria (eq 1 and 2) which do not involve any change in the number of reactant and product molecules are characterized by large $\Delta S°$ values.[4] We conclude, therefore, that the $\Delta S°$ and $\Delta S^‡$ values for the reactions of eq 1 and 2 arise primarily from the solvation characteristics of the reactants, transition states, and products.

Structure of the Transition State for the Nonenzymatic Reaction. The substituent effects with N-1-substituted 1,4-dihydronicotinamides on the rates of the uncatalyzed reduction of PCA show that the ring nitrogen atom of the reductant is fully charged in the transition state, which, on this basis, is very product-like (Srinivasan et al., 1982). The primary kinetic isotope effect of 1.3, obtained with NADH and 4,4-dideuterio-NADH, is also consistent with such a product-like transition state (Srinivasan & Fisher, 1985b). However, the reaction (eq 1) is exergonic. It follows that the transition-state structure of the uncatalyzed PCA reaction could not be deduced simply by the application of Hammond's postulate (Hammond, 1955) and that it has to be elucidated empirically.

Since the solvent reorganization is best expressed in the entropies of activation or reaction, $\Delta S^‡$ values are often compared to $\Delta S°$ values to determine the solvation characteristics of the transition state (Arnett & Reich, 1980; Schaleger & Long, 1963). The entropy of activation for the reduction of the iminium ion by NADPH and the standard entropy change for the same reaction are −5 ± 3 and 33 ± 4 eu, respectively, indicating that solvent reorganization has hardly begun at the transition state.

Kreevoy & Lee (1984) suggested that the in-flight hydrogen in a hydride transfer reaction should bear a partial negative charge. In fact, Bunting & Sindhuatmadja (1981), who studied the redox rates with substituted oxidants and reductants in an analogous hydride transfer reaction, found that the in-flight hydrogen bears a charge of −0.44 e in the transition state. It is quite likely that the in-flight hydrogen in the transition state of the uncatalyzed PCA reaction also possesses such partial negative charge. We conclude that the reduction of the imino acid by the reduced coenzyme proceeds through a transition state that is very product-like with respect to the dihydronicotinamide, somewhat product-like with respect to the imino acid, and very reactant-like with respect to its solvation properties. These features for the transition state are reminiscent of those for the transition state of the Menschutkin reaction (Arnett & Reich, 1980).

Structure of the Transition State for the Enzymatic Reaction. It is interesting to note that the reduction of PCA by E–NADPH shows no apparent deviation from Hammond's postulate (Hammond, 1955). The $\Delta G°$ value of −3.1 kcal/mol for this reaction predicts a fairly symmetric transition state. The primary kinetic deuterium isotope effect for the reduction of the imino acid by NADH and [4,4-^{2}H]NADH is 1.3 while that for the reduction of the same imino acid by the enzyme–NADH and enzyme–[4,B-^{2}H]NADH complexes is 3.8 (Srinivasan & Fisher, 1985b). Thus, the primary isotope effect data also point to a more symmetric transition state for the enzymatic reaction. The comparison of a $\Delta S^‡$ of 14 eu for the reduction of PCA by E–NADPH with a $\Delta S°$ of 27 eu for the same reaction in eq 2 suggests that the extent of solvation of the transition state is indeed midway between those of the reactants and the products.

Free Energy Course of the Enzymatic Reaction. It is apparent from the free energy profiles shown in Figure 2 that the binding of the enzyme to the coenzyme actually lowers the "catalytic free energy" (Schowen, 1978)—the difference in the free energies of activation for the reductions by NADPH and E–NADPH—although the formation of the enzyme–coenzyme complex is exergonic. The enzyme has utilized the favorable binding energy[5] in forming E–NADPH to gain considerable entropic advantage of the ensuing steps (which

[3] Since the transition states are to be considered to exist at the maxima of the free energy and not of the entropy barrier (Laidler, 1969), the latter may indeed be higher at other points along the reaction coordinate.

[4] The $\Delta S°$ value of 27 eu for the reaction in eq 2 might arise from the difference in solvation of E–NADPH and E–NADP$^+$ since the dissociation constants of several enzyme groups are different in these complexes (Rife & Cleland, 1980). However, it is not clear why $\Delta S°$ for the reaction in eq 1 is a large positive number. The transfer of the positive charge from PCA to the oxidized coenzyme where it is highly delocalized may in part be responsible for the observed $\Delta S°$ value. It is possible that the solvation of the iminium ion involves several water molecules (Schaleger & Long, 1963; Taft et al., 1978) that are virtually freed upon formation of the oxidized coenzyme.

[5] We do not find it useful to discuss the catalysis of the model reaction in terms of the enthalpy and entropy of formation of E–NADPH since the formation of the binary complex in 0.1 M phosphate buffer is enthalpy driven and entropy opposed at pH 8.85 (unpublished results from the laboratory) while the converse is true at pH 7.60 (Subramanian et al., 1978).

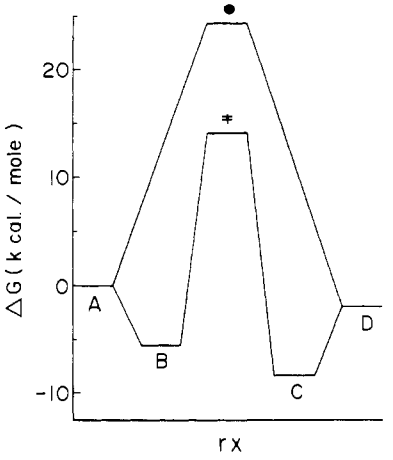


FIGURE 2: Free energy reaction coordinate diagram for the nonenzymatic (upper profile) and enzymatic reactions (lower profile) in 0.1 M Tris–0.01 M phosphate–1 M proline–0.05 M PCA buffer at pH 8.85 and at 25 °C. The free energy of activation for the formation of the enzyme–coenzyme complexes is not shown. The standard free energy of formation of the enzyme–coenzyme–substrate complexes is also not shown; they are present at undetectably low concentrations under the experimental conditions. See text regarding positions of transition states. A = E, PCA, and NADPH; B = PCA and E–NADPH; C = proline and E–$NADP^+$; D = E, proline, and $NADP^+$.

involve at least the formation of the E–NADPH–PCA complex and the subsequent redox step).

Comparison to the Glutamate Reaction. Although the chemical steps are not rate contributing during the steady-state oxidative deamination of L-glutamate (Iwatsubo & Pantaloni, 1967; Colen et al., 1975), the hydride transfer step is at least partly rate limiting under transient-state conditions (Fisher et al., 1970). Colen et al. (1981) have studied the transient-phase energetics of the glutamate reaction and found that the entropy barrier of the transient-phase phenomenon is considerably smaller than its enthalpy barrier. The data for the L-proline ~ PCA reaction, which is a model for the L-glutamate ~ α-iminoglutarate reaction, demonstrate that the enzyme achieves catalysis of the model reaction entirely by minimizing the entropy barrier. We suggest that glutamate dehydrogenase may catalyze the redox step of the glutamate reaction in an analogous way.

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