Schachman, H. K. (1959) *Ultracentrifugation in Biochemistry*, p 82, Academic Press, New York.
Sober, H. A., Ed. (1970) *Handbook of Biochemistry*, 2nd ed., pp C-67-69, Chemical Rubber Publishing Co., Cleveland, OH.
Squire, P. G., & Li, C. H. (1961) *J. Am. Chem. Soc. 83*, 3521-3528.
Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp 194-233, Wiley, New York.
Teller, D. C. (1973) *Methods Enzymol. 27*, 346-441.
Teller, D. C., Horbett, T. A., Richards, E. G., & Schachman, H. K. (1969) *Ann. N.Y. Acad. Sci. 64*, 66-101.
Van Holde, K. E., & Baldwin, R. I. (1958) *J. Phys. Chem. 62*, 734-743.
Washburn, E. W., Ed. (1928) *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*, Vol. 3, McGraw-Hill, New York.
Weast, R. C., Ed. (1968) *Handbook of Chemistry and Physics*, 49th ed., Chemical Rubber Publishing Co., Cleveland, OH.
Weber, G. (1975) *Adv. Protein Chem. 29*, 1-83.
Yphantis, D. A. (1964) *Biochemistry 3*, 297-317.
Yphantis, D. A., & Waugh, D. F. (1956) *J. Phys. Chem. 60*, 623-629.
Yphantis, D. A., Correia, J. J., Johnson, M. L., & Wu, G.-M. (1978) in *Physical Aspects of Protein Interactions* (Castimpoolas, N., Ed.) pp 275-303, Elsevier/North-Holland, New York.

Octopine Dehydrogenase from *Pecten maximus*: Steady-State Mechanism†

Jeffrey L. Schrimsher‡ and Kenneth B. Taylor*

ABSTRACT: The steady-state kinetic mechanism of the reaction catalyzed by octopine dehydrogenase [N^2-(1-carboxyethyl)-L-arginine:NAD^+ oxidoreductase] was investigated at pH 6.9 and 9.2 by studies of substrate inhibition, analogue inhibition, and product inhibition. In the direction of octopine synthesis, the inhibition patterns in the presence of δ-guanidinovalerate and propionate show that NADH binds to the enzyme first followed by L-arginine and pyruvate which bind randomly. In the direction of octopine oxidation, the substrate patterns show that NAD binds to the enzyme before octopine in a rapid equilibrium fashion, and the product inhibition patterns show that the products L-arginine and pyruvate are released in a random fashion. Double, synergistic, substrate inhibition by L-arginine and pyruvate was shown to be due to binding (hypothetically of the imine) to the free enzyme and the enzyme–NAD complex. Furthermore, an alternate minor pathway was demonstrated which includes an enzyme–NADH–octopine complex and an enzyme–octopine complex.

Octopine dehydrogenase from *Pecten maximus* (EC 1.5.1.11) catalyzes the reaction

L-arginine + pyruvic acid + NADH + H^+ ⇌ D(+)-octopine + NAD^+ + H_2O

The enzyme is a monomer with a molecular weight of 38 000 (Thoai et al., 1969), and although it lacks any metal or flavin (Thoai et al., 1969; Pho et al., 1970), it contains a prosthetic group involved in electron transfer (J. L. Schrimsher and K. B. Taylor, unpublished results) whose structure is not presently known.

Several kinetic studies of octopine dehydrogenase have been undertaken (Doublet & Olomucki, 1975; Monneuse-Doublet et al., 1978), but these studies were limited by substrate inhibition to a rather narrow range of substrate concentrations and lacked the use of statistical analysis of data.

Octopine dehydrogenase has also been shown to exhibit substrate inhibition by L-arginine and pyruvate as well as octopine [(*R*)-N^2-(1-carboxyethyl)-L-arginine] (Monneuse-Doublet et al., 1978). However, the mechanism of the substrate inhibition was not examined.

The following reports our investigation on the nature of the substrate inhibition induced by L-arginine and pyruvic acid, the mechanism of several reversible, analogue inhibitors, and the steady-state kinetic mechanism of octopine dehydrogenase from *Pecten maximus*.

Experimental Procedures

Materials. L-Arginine hydrochloride, sodium pyruvate, NAD, and NADH were from Sigma Chemical Co. Propionic acid was from J. T. Baker Chemical Co. D(+)-Octopine was a generous gift of Dr. Leo Hall, University of Alabama in Birmingham.

Octopine dehydrogenase from *Pecten maximus*, obtained as a dry powder from Sigma Chemical Co., had a specific activity of 11.2 units/mg of protein. The results of polyacrylamide gel electrophoresis indicate that the enzyme preparation is homogeneous (J. L. Schrimsher and K. B. Taylor, unpublished results).

Preparation of Inhibitors. δ-Guanidinovalerate was prepared from δ-aminovalerate (3.1 g) according to the procedure described by Kurtz (1949) for the synthesis of arginine from ornithine except that the steps for the protection of the α-amine were excluded. The pH of the solution containing the product was adjusted to 1.0 with HCl, and the solution was applied (1 mL/min, 55 mL) to a column (1.6 × 13 cm) of Dowex 50W-X8 (H^+ form). The column was developed with 0.1 M ammonia, and the effluent that contained product [detected according to the procedure of Otten & Schilperoort (1977)] was dried in vacuo. The product (2.3 g) was recrystallized twice from water (20 mL). N^2-Ethyl-L-arginine was synthesized from L-arginine and acetaldehyde according to the procedure of Biellmann et al. (1977) for reductive amination

† From the Department of Biochemistry, University of Alabama in Birmingham, Birmingham, Alabama 35294. *Received August 25, 1983.*
‡ Present address: Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

0006-2960/84/0423-1348$01.50/0

Table I: Substrate and Substrate Inhibition Patterns for the Reaction Catalyzed by Octopine Dehydrogenase

variable substrate	other substrates	substrate pattern	substrate inhibition type	K_A (mM)	K_B (mM)	K_{ia} (mM)	K_{ib} (mM)
L-arginine[a]	pyruvate (0.2-40 mM), NADH (0.2 mM)	INT[c]	UC[d]	1.96 ± 0.07	1.01 ± 0.07		
NADH[a,b]	L-arginine (0.2-40 mM), pyruvate (10 mM)[g]	INT	NC[e]	0.037 ± 0.004	2.2 ± 0.3	0.038 ± 0.004[h]	2.3 ± 0.5
NADH[a,b]	pyruvate (0.2-40 mM), L-arginine (20 mM)[g]	INT	NC	0.017 ± 0.001	1.0 ± 0.1	0.031 ± 0.004	1.2 ± 0.3
NAD[b]	octopine (0.2-10.5 mM)	EO[f]			1.5 ± 0.1	0.67 ± 0.06	

[a] Experiment done at pH 6.9. [b] Experiment done at pH 9.2. [c] INT, interesting to the left of the vertical axis (from data of noninhibitory substrate levels, fitted to eq 2). [d] UC, uncompetitive. [e] NC, noncompetitive. [f] EO, equilibrium ordered (fitted to eq 3). [g] Patterns of substrate inhibition by pyruvate and arginine are with respect to the variable substrate. [h] Substrate a is NADH and NAD in their respective experiments.

with sodium cyanoborohydride.

Initial Velocity Measurement. The reactions catalyzed by octopine dehydrogenase were monitored by the absorbance of NADH at 340 nm in a Beckman monochromator connected to a Gilford optical density converter, and the results were displayed on a strip chart recorder. Cuvettes with a 1.0-cm light path contained 1 mL of reaction mixture (30 ± 0.1 °C). Reactions were initiated by the addition of enzyme to the cuvettes which had been preincubated (30 °C for at least 10 min) with the substrates and buffer.

Assays in the direction of octopine synthesis were conducted in 0.1 M potassium phosphate (pH 6.9) unless otherwise noted. The final enzyme concentration was 3 μg/mL), except in the study of substrate inhibition where the concentration of octopine dehydrogenase was 5 μg/mL. The concentration ranges of the substrates and inhibitors were as follows: L-arginine and pyruvate, from 0.2 to 40 mM; NADH, from 0.01 to 0.3 mM; octopine, from 0.5 to 40 mM; δ-guanidinovalerate, from 0 to 0.15 mM; propionate, from 0 to 100 mM.

Assays in the direction of octopine oxidation were conducted in 50 mM 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) (pH 9.2), and the final concentration of enzyme was 40 μg/mL. The concentrations of the substrates and inhibitors were varied as follows: octopine, from 0.2 to 50 mM; NAD, from 0.018 to 1.0 mM; N^2-ethyl-L-arginine, from 20 to 150 mM; L-arginine and pyruvate, from 0.2 to 20 mM.

Data Analysis. Data from experiments in which the concentration of only a single substrate was varied were fitted to eq 1 for determination of slopes and intercepts. K_A is the K_m

$$v = VA/(K_A + A) \quad (1)$$

value of substrate A. Data for intersecting double-reciprocal plots from experiments in which the concentration of two substrates was varied were fitted to eq 2 or eq 3, where K_{ia}

$$v = VAB/(K_{ia}K_B + K_AB + K_BA + AB) \quad (2)$$

$$v = VAB/(K_{ia}K_B + K_BA + AB) \quad (3)$$

is the dissociation constant of substrate A and K_A and K_B are the K_m values for substrates A and B, respectively. Data from dead-end or product inhibition experiments were fitted to eq 4–6 for competitive, noncompetitive, and uncompetitive pat-

$$v = VA/[K_A(1 + I/K_{is}) + A] \quad (4)$$

$$v = VA/[K_A(1 + I/K_{is}) + A(1 + I/K_{ii})] \quad (5)$$

$$v = VA/[K_A + A(1 + I/K_{ii})] \quad (6)$$

terns, respectively, where K_{is} and K_{ii} are the dissociation constants of the inhibitor from the enzyme form which affect the slopes or the intercepts, respectively. Replots of slopes or intercepts were fitted to eq 7 for linear replots or to eq 8 for parabolic replots, where *a*, *b*, and *c* are constants:

$$y = aA + b \quad (7)$$

$$y = a + bA + cA^2 \quad (8)$$

Data demonstrating substrate inhibition were fitted to eq 9, and the apparent K_I (K_{Ia})[1] values were fitted to eq 10 for cases

$$v = VA/(K_A + A + A^2/K_{Ia}) \quad (9)$$

$$K_{Ia} = a/B \quad (10)$$

of synergistic substrate inhibition, where *a* is a constant. The type of substrate inhibition was determined as described by Cleland (1979b) from replots of the slopes and intercepts of the double-reciprocal plots from experiments in which a noninhibitory substrate was the variable substrate and the inhibitory substrate was the constant variable substrate. In cases of double substrate inhibition, the slopes and intercepts were obtained from the fit of the data to eq 9, whereas in cases in which only one substrate was inhibitory, the slopes and intercepts were obtained from the fit of the data to eq 1.

The computer programs of Cleland (1979a) were used to fit the data to eq 1–6 and 9. Least-squares programs, written by ourselves, were used to fit data to eq 7, 8, and 10. The best fit of the data, relative to other models, was determined by the *F* test, by comparison of the sums of the squares of the residuals, or by comparison of the standard errors of the determined parameters.

Results

Initial Velocity Studies in the Enzymatic Synthesis of Octopine. When the substrates (L-arginine, pyruvate, and NADH) were varied at noninhibitory levels with respect to each other, the double-reciprocal plots of the data intersected to the left of the vertical axis in all cases (Table I), in contrast to a previous investigation (Doublet & Olomucki, 1975) where parallel lines were obtained when L-arginine was varied with respect to NADH in the presence of a high concentration of pyruvate. The dissociation constants and K_m values for the substrates of octopine dehydrogenase are given in Table I.

The results of the experiments in which the concentrations of the inhibitors, either propionate or δ-guanidinovalerate, were varied with respect to each substrate (pH 6.9) are summarized in Table II. The inhibition patterns at both pH 6.9 and 9.2

[1] In order to distinguish the latter from K_{ia}, the substrate inhibition constant is designated K_I, and the apparent substrate inhibition constant is designated K_{Ia}.

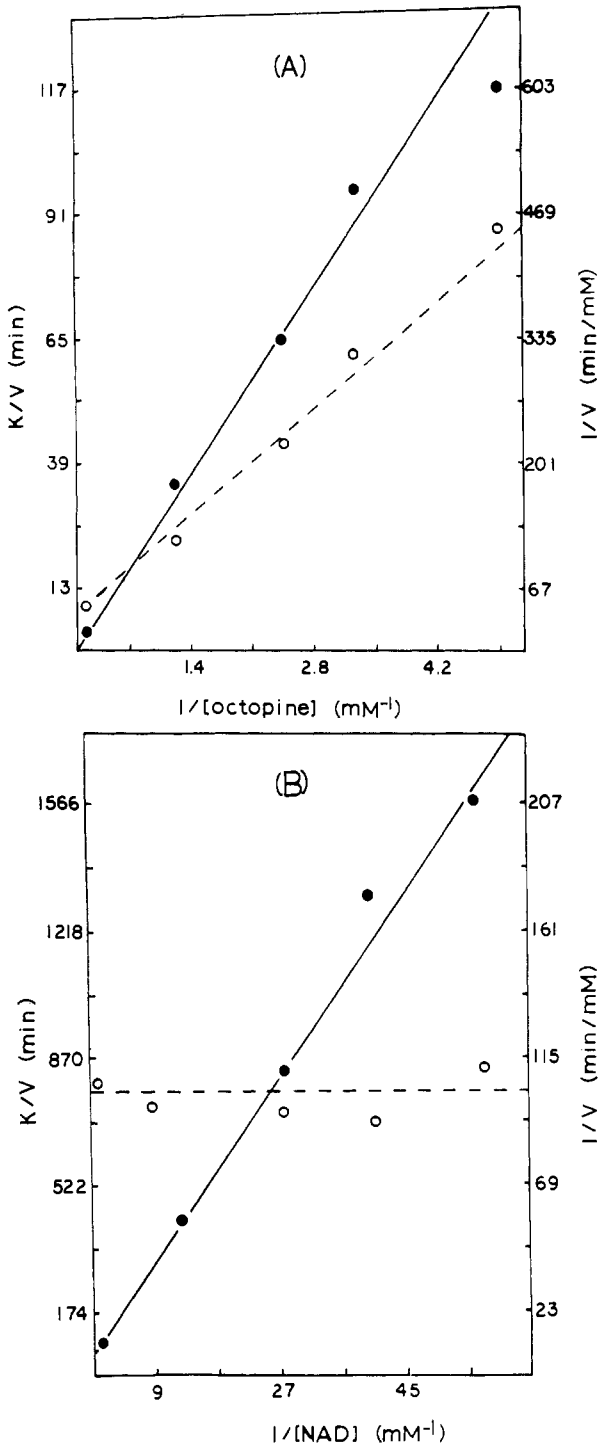

FIGURE 1: Replots of the slopes (●) and intercepts (○) vs. the reciprocal concentration of octopine with NAD as the variable substrate (A) and vs. the reciprocal concentration of NAD with octopine as the variable substrate (B). All assays were conducted in 50 mM CHES (pH 9.2, 30 °C) and contained 0.04 mg/mL octopine dehydrogenase. The data were fitted to eq 3 to obtain the experimental points and to eq 7 to obtain the predicted lines.

(δ-guanidinovalerate only) are the same as those described by Fromm (1979a) in which one substrate binds first and the other two bind randomly (Fromm, 1979b). Thus, NADH binds before L-arginine or pyruvate, but L-arginine and pyruvate bind to the enzyme–NADH complex randomly, rather than in an ordered fashion as was previously hypothesized by Doublet & Olomucki (1975).

The results of the experiments with propionate and δ-guanidinovalerate also eliminate the possibility that the imine formed by L-arginine and pyruvic acid is the sole species that

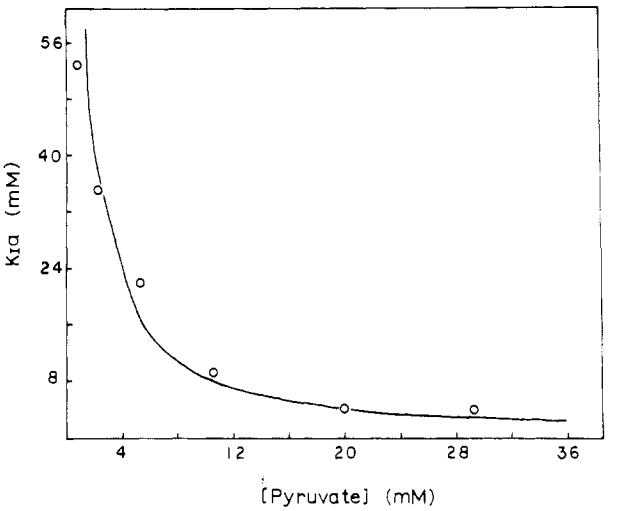

FIGURE 2: Plot of the apparent K_I (K_{Ia}), determined from the substrate inhibition by L-arginine, vs. the concentration of pyruvate. All assays were performed in 0.1 M potassium phosphate (pH 6.9, 30 °C) in the presence of 0.005 mg/mL octopine dehydrogenase and 0.2 mM NADH. The concentration of L-arginine was varied from 0.2 to 40 mM. The data were fitted to eq 9 for determination of each K_{Ia}. The calculated line was determined from eq 10.

binds productively to the enzyme–NADH complex. If this were the case, competitive inhibition should have been observed with either δ-guanidinovalerate with respect to pyruvate or propionate with respect to L-arginine. However, the possibility that the imine will also bind productively to the enzyme–NADH complex cannot be excluded.

Initial Velocity Studies of the Enzymatic Oxidation of Octopine. The nonlinearity described by Monneuse-Doublet et al. (1978) at pH 7.0 when NAD was the variable substrate was not observed under the conditions of our assays. When NAD was the variable substrate, the double-reciprocal plots with different concentrations of octopine intersect to the left of the vertical axis, and the slope replot goes through the origin (Figure 1A). With octopine as the variable substrate, the intercept replot is horizontal (Figure 1B). These two patterns are characteristic of a model in which NAD binds first in a rapid equilibrium step with respect to V, followed by the binding of octopine (Cleland, 1977). The fact that dead-end inhibition by N^2-ethyl-L-arginine is competitive with respect to either NAD or octopine (Table II) indicates that it forms a dead-end complex only with the free enzyme, under the conditions of the experiments.

Substrate Inhibition. In a previous investigation, Monneuse-Doublet et al. (1978) reported that pyruvate and L-arginine showed substrate inhibition. However, no kinetic investigation of this inhibition was reported. The complete substrate inhibition by pyruvate was demonstrated by the linearity of the plot of the concentration of pyruvate vs. the reciprocal of the initial velocity at 20 mM L-arginine. The relationship between the concentration of pyruvate and the apparent K_I for substrate inhibition by L-arginine (Figure 2) and the complementary relationship for pyruvate inhibition (not shown) shows that the substrate inhibition by each substrate is synergistic with the other. This synergism suggests either formation of an inhibitory complex between pyruvate and L-arginine (e.g., the imine) or highly synergistic separate binding.

The fact that the substrate inhibition observed at inhibitory levels of L-arginine or pyruvate with respect to noninhibitory levels of pyruvate or L-arginine, respectively, is uncompetitive [Table I (pH 6.9)] indicates that the inhibitory complex does not bind to any of the enzyme forms that bind L-arginine or

Table II: Inhibition Patterns and Inhibition Constants Obtained for Dead-End Inhibitors of the Reaction Catalyzed by Octopine Dehydrogenase

variable substrate	constant substrate(s)	inhibitor	inhibition type[a]	K_{ii} (mM)	K_{is} (mM)
L-arginine[b,c]	pyruvate (4.0 mM), NADH (0.2 mM)	δ-guanidinovalerate	C		0.019 ± 0.002
pyruvate[b,c]	L-arginine (2.0 mM), NADH (0.2 mM)	δ-guanidinovalerate	NC	0.036 ± 0.006	0.036 ± 0.006
NADH[b,c]	L-arginine (4.0 mM), pyruvate (4.0 mM)	δ-guanidinovalerate	UC	0.054 ± 0.004	
L-arginine[b]	pyruvate (1.0 mM), NADH (0.2 mM)	propionate	NC	27.9 ± 2.4	50.9 ± 6.6
pyruvate[b]	L-arginine (4.0 mM), NADH (0.2 mM)	propionate	C		11.4 ± 1.5
NADH[b]	L-arginine (2.0 mM), pyruvate (1.0 mM)	propionate	UC	24.4 ± 2.4	
octopine[c]	NAD (0.5 mM)	L-arginine	NC	4.7 ± 0.3	7.7 ± 1.3
octopine[c]	NAD (0.5 mM)	pyruvate	NC	2.5 ± 0.5	0.51 ± 0.16
octopine[c]	NAD (0.1 mM)	*N*-ethyl-L-arginine	C		69 ± 5
NAD[c]	octopine (2.0 mM)	*N*-ethyl-L-arginine	C		51 ± 6
NAD[c]	octopine (2.0 mM)	NADH	C		0.044 ± 0.004

[a] Abbreviations: C, competitive; NC, noncompetitive; UC, uncompetitive. [b] pH 6.9, 30 °C. [c] pH 9.2, 30 °C.

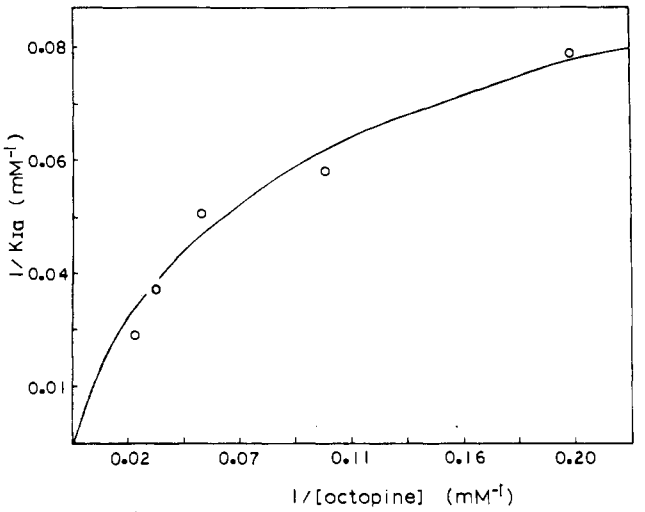

FIGURE 3: Double-reciprocal plot of the apparent K_I (K_{Ia}) of pyruvate vs. the concentration of octopine as a product inhibitor. Assays were conducted on 0.1 M potassium phosphate (pH 6.9, 30 °C) and contained 0.04 mg/mL octopine dehydrogenase, 0.05 mM NADH, and 20 mM L-arginine. The data were fitted to eq 9 to obtain each K_{Ia}.

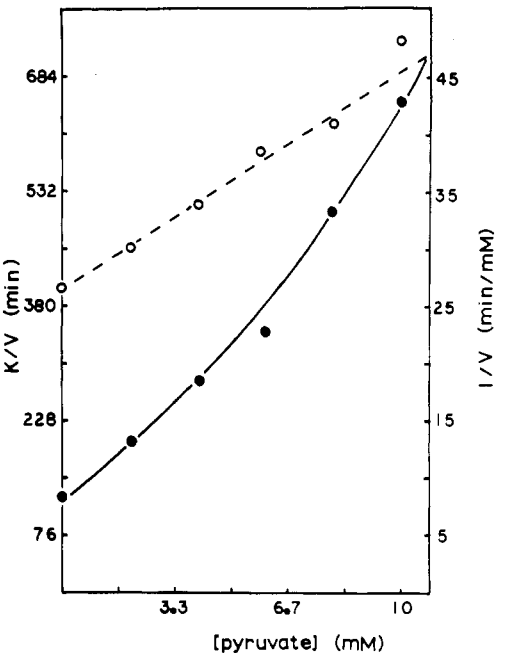

FIGURE 4: Replot of the slopes (●) and intercepts (○) of the product inhibition by pyruvate in the presence of 1.32 mM L-arginine with octopine as the variable substrate. Assays were conducted in 50 mM CHES (pH 9.2, 30 °C) and contained 0.04 mg/mL octopine dehydrogenase and 0.1 mM NAD. The data were fit to eq 1 for determination of experimental slopes and intercepts, to eq 8 for the predicted parabola, and to eq 7 for the predicted line.

pyruvate and that it binds later in the cycle to an enzyme form separated from enzyme–NADH by at least one irreversible step (e.g., NADH binding). Furthermore, the noncompetitive substrate inhibition vs. NADH (Table I, determined at pH 6.9 and 9.2) indicates that the inhibitor binds either to two enzyme forms or to one form reversibly connected with the enzyme form that binds NADH. The fact that NADH binds prior to L-arginine and pyruvate and that there are no irreversible steps between NADH binding and the binding of L-arginine and pyruvate indicates that the inhibitory complex binds competitively to the enzyme form that binds NADH and also to some enzyme–product complex.

Furthermore, the fact that reciprocals of the apparent K_I values, obtained from the substrate inhibition experiments by pyruvate in the presence of octopine, approach zero when the concentration of octopine becomes high (Figure 3) shows that octopine can prevent the inhibition completely. Therefore, octopine must bind to the same enzyme forms as the inhibitor. The results of the substrate inhibition experiments and the effect of octopine on the apparent K_I of the inhibitory complex show that octopine can bind to free enzyme.

Substrate inhibition by octopine itself (pH 9.2) was found to occur at levels greater that 15 mM. However, the nonlinearity of this inhibition (not shown) indicates a productive, but minor, alternate pathway in which octopine, at high concentrations, binds to either the enzyme–NADH complex, the free enzyme, or both. Substrate inhibition by octopine has been demonstrated previously at pH 7.0 (Monneuse-Doublet et al., 1978).

Product Inhibition Studies. The noncompetitive product inhibition by both L-arginine and pyruvate with respect to octopine (Table I) indicates the random release of these products. In addition, the replots of the slopes and intercepts were linear in the case of both product inhibitors. However, when the product inhibition by either pyruvate or L-arginine was repeated in the presence of a constant amount of the other,

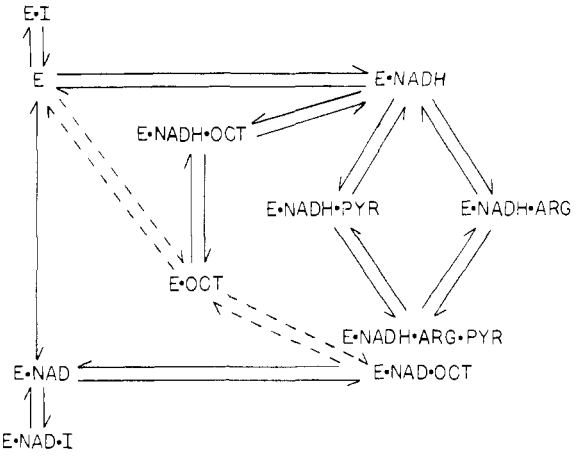

FIGURE 5: Schematic representation of the reaction catalyzed by octopine dehydrogenase. Symbols are used to represent octopine dehydrogenase (E), L-arginine (ARG), pyruvate (PYR), the imine inhibitor (I), and octopine (OCT). The dashed lines represent alternate pathways.

the replots of the slopes become parabolic in both cases (Figure 4), whereas the replots of the intercepts remain linear. This effect on the slopes indicates that the inhibitory complex formed between L-arginine and pyruvate binds only the enzyme forms that bind octopine, i.e., the enzyme–NAD complex, free enzyme, or both.

At 2 mM octopine, NADH was found to be a competitive product inhibitor with respect to NAD at pH 9.2 (Table I). However, at 20 mM octopine, the nonlinear double-reciprocal plots from the same experiment are consistent with the existence of an enzyme–NADH–octopine complex that is slowly productive (probably by dissociation of NADH).

Steady-State Mechanism. Figure 5 shows the steady-state mechanism that is consistent with the results of the preceding experiments.

Discussion

The results presented above show that in the synthesis of octopine, catalyzed by octopine dehydrogenase, NADH binds to the enzyme before L-arginine and pyruvate and that L-arginine and pyruvate bind to the enzyme–NADH complex in a random fashion. Previously, Doublet & Olomucki (1975) concluded that the binding of pyruvate and L-arginine to the enzyme–NADH complex is ordered with pyruvate binding before L-arginine, on the basis of the observation that the double-reciprocal plots were parallel in an experiment in which NADH and L-arginine were varied at a constant concentration of pyruvate, 4 mM, but not at lower pyruvate concentrations. However, it is likely that the substrate inhibition by L-arginine prevented the use of a sufficient concentration range of that substrate to distinguish between parallel and intersecting patterns. Therefore, we have studied dead-end inhibition to distinguish between these models.

In analogy to the reaction catalyzed by octopine dehydrogenase, glutamate synthase also catalyzes a reductive amination. In the course of the reaction, the substrates, 2-oxoglutarate and L-glutamine, bind to the enzyme in a random fashion (Rendina & Orme-Johnson, 1978). However, the order of binding of lysine and 2-oxoglutarate to saccharopine dehydrogenase (EC 1.5.1.7) seems to be strictly ordered (Fujioka, 1974).

In the enzymatic oxidation of octopine, NAD dissociates from the enzyme–NAD complex faster than that complex goes on to product (rapid equilibrium binding). Octopine binds to the enzyme–NAD complex more tightly than to free enzyme, but it does bind significantly to the latter (particularly at high concentrations). Therefore, the mechanism is synergistic rather than completely ordered. The fact that *N*-ethylarginine binds more tightly to free octopine dehydrogenase than to the enzyme–NAD complex indicates that the carboxyl group from pyruvate is critical in the synergistic binding of octopine. The dissociation constant of NAD obtained in this study is similar to that obtained in a previous fluorometric study (0.4 mM, pH 9.0, 22 °C) by Luisi et al. (1973). However, Doublet & Olomucki (1975) did not demonstrate rapid equilibrium binding of NAD to enzyme under steady-state conditions.

The hypothesis of a degree of randomness is in agreement with previous data (J. L. Schrimsher and K. B. Taylor, unpublished results) in which octopine dehydrogenase was shown to oxidize octopine in the absence of nucleotide. Such an alternate pathway has previously been demonstrated both for L-alanine dehydrogenase (Grimshaw & Cleland, 1981), where L-alanine may bind before NAD, and for glutamate dehydrogenase (Cohen et al., 1977; Rife & Cleland, 1980), where L-glutamate may bind before NADP. The enzyme–NADH–octopine complex, demonstrated by nonlinear double-reciprocal plots of NAD in the presence of NADH, was previously demonstrated in a fluorometric study of octopine dehydrogenase (Luisi et al., 1973).

Product inhibition by either pyruvate or L-arginine resulted in noncompetitive patterns with respect to octopine. This result shows that the release of L-arginine and pyruvate is also random. The results of this product inhibition study are consistent with the results of a previous study (Doublet & Olomucki, 1975). However, in the previous report, no explanation was offered for these results.

The double, synergistic substrate inhibition by L-arginine and pyruvate is due to the formation of dead-end complexes with both the enzyme–NAD complex and the enzyme form that binds reduced nucleotide. The synergistic phenomenon can be explained as either the formation of an inhibitory complex on the enzyme, in which the binding of one substrate (L-arginine or pyruvate) induces the binding of the other, or the prior formation of an inhibitory species between L-arginine and pyruvate, which binds to the enzyme. The fact that the product inhibition by L-arginine or pyruvate with respect to octopine is linear in both slopes and intercepts unless both are present indicates that neither L-arginine nor pyruvate alone can bind to the enzyme forms to which inhibitor binds, under the experimental conditions. This supports the hypothesis that the inhibitory species that binds to the enzyme is the imine formed by L-arginine and pyruvate. The imine is structurally similar to the product octopine and to a probable reaction intermediate. Substrate inhibition is found to occur in other enzyme-catalyzed reductive aminations. Saccharopine dehydrogenase (Fujioka, 1975), L-alanine dehydrogenase (Grimshaw & Cleland, 1981), and glutamate dehydrogenase (Rife & Cleland, 1980) all exhibit substrate inhibition with both the α-keto acid substrate and the amine substrate. Grimshaw & Cleland (1981) have proposed that the imine formed between ammonia and pyruvate is the species which binds to L-alanine dehydrogenase. However, the synergistic nature of the substrate inhibition of the other enzymes has not been demonstrated.

Octopine dehydrogenase from crown gall tumor tissue catalyzes a reaction similar to that catalyzed by octopine dehydrogenase from *Pecten maximus* (Otten et al., 1977). The crown gall tumor enzyme was also observed to exhibit substrate inhibition (Hack & Kemp, 1980), and it was concluded that the substrates, L-arginine and pyruvate, bind to the enzyme–

NADPH complex randomly. It will be interesting to see if the crown gall tumor enzyme exhibits other similarities in its catalytic mechanism to octopine dehydrogenase from *Pecten maximus*.

Acknowledgments

We thank Dr. Ted Sakai of the University of Alabama in Birmingham for his assistance in the preparation of the inhibitors used in this study.

Registry No. NADH, 58-68-4; NAD, 53-84-9; L-arginine, 74-79-3; pyruvate, 127-17-3; octopine, 34522-32-2; δ-guanidinovalerate, 462-93-1; propionate, 79-09-4; N^2-ethyl-L-arginine, 88855-11-2; octopine dehydrogenase, 37256-27-2.

References

Biellmann, J. F., Branlant, G., & Wallen, L. (1977) *Bioorg. Chem. 6*, 89.
Cleland, W. W. (1970) *Enzymes, 3rd Ed. 2*, 1.
Cleland, W. W. (1977) *Adv. Enzymol. Relat. Areas Mol. Biol. 45*, 273.
Cleland, W. W. (1979a) *Methods Enzymol. 64*, 103.
Cleland, W. W. (1979b) *Methods Enzymol. 64*, 500.
Cohen, A. H., Wilkinson, R. R., & Fisher, H. F. (1977) *Biochim. Biophys. Acta 481*, 377.
Doublet, M.-O., & Olomucki, A. (1975) *Eur. J. Biochem. 59*, 175.
Fromm, H. J. (1979a) *Methods Enzymol. 63*, 467.
Fromm, H. J. (1979b) *Methods Enzymol. 63*, 42.
Fujioka, M. (1975) *J. Biol. Chem. 250*, 8986.
Greenstein, J. P., & Winitz, M. (1961) in *Chemistry of the Amino Acids*, Vol. 3, pp 1849–1850, Wiley, New York.
Grimshaw, C. E., & Cleland, W. W. (1981) *Biochemistry 20*, 5650.
Hack, E., & Kemp, J. D. (1980) *Plant Physiol. 65*, 949.
Kurtz, A. C. (1949) *J. Biol. Chem. 180*, 1253.
Luisi, P. L., Olomucki, A., Baici, A., & Karlovic, D. (1973) *Biochemistry 12*, 4100.
Monneuse-Doublet, M.-O., Olomucki, A., & Buc, J. (1978) *Eur. J. Biochem. 84*, 441.
Otten, L. A. B. M., Vreugdenhil, D., & Schilperoort, R. A. (1977) *Biochim. Biophys. Acta 485*, 268.
Pho, D. B., Olomucki, A., Huc, C., & Thoai, N. V. (1970) *Biochim. Biophys. Acta 206*, 46.
Rendina, A. R., & Orme-Johnson, W. H. (1978) *Biochemistry 17*, 5388.
Rife, J. E., & Cleland, W. W. (1980) *Biochemistry 19*, 2321.
Thoai, N. V., Huc, C., Pho, D. B., & Olomucki, A. (1969) *Biochim. Biophys. Acta 191*, 46.

Activation of an Erythrocyte NAD:Arginine ADP-Ribosyltransferase by Lysolecithin and Nonionic and Zwitterionic Detergents†

Joel Moss,* James C. Osborne, Jr., and Sally J. Stanley

ABSTRACT: The activity of an NAD:arginine ADP-ribosyltransferase was stimulated 4–6-fold by lysolecithin; lysolecithins containing long-chain fatty acids such as stearoyl (C_{18}) and palmitoyl (C_{16}) were more effective than those with shorter chains: $C_{14} > C_{12} > C_{10} \simeq C_8$. The analogue lacking a fatty acid at C-1, α-glycerophosphocholine, was inactive as were choline, lysophosphatidic acid, lysophosphatidylserine, lysophosphatidylglycerol, lysophosphatidylethanolamine, lecithin, phosphatidic acid, phosphatidylserine, and phosphatidylethanolamine. Activation of the transferase was, however, also observed with certain nonionic (e.g., Triton X-100) and zwitterionic [3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate] detergents. The transferase was shown previously to be stimulated by chaotropic salts or histones; in the presence of maximally effective concentrations of lysolecithin, salt, and histone, the activity was similar to that observed in the presence of histone or salt alone. Maximal activation by lysolecithin and detergents was less than that observed with either salt or histone. It appears that activation by lysolecithin shows significant differences from that observed previously with histones or salt and can be mimicked by certain nonionic and zwitterionic detergents.

Covalent modification of proteins plays a critical role in the biological function of many proteins and enzymes. For instance, the transfer of the ADP-ribose moiety of NAD to the regulatory component of adenylate cyclase or to elongation factor II of the protein synthetic pathway appears to be critical to the action of choleragen (cholera toxin) and diphtheria toxin, respectively (Moss & Vaughan, 1979; Pappenheimer, 1977). In the reactions catalyzed by these toxins, it appears that a single ADP-ribose moiety is placed on a critical amino acid. In animal tissues, two distinct types of ADP-ribosyltransferases have been described: one enzyme, poly(ADP-ribose) synthetase, may catalyze both the initial ADP-ribosylation of protein and also the subsequent addition of ADP-ribose moieties to form a polymeric structure (Hayaishi & Ueda, 1977; Pekala & Moss, 1983). A second type, a mono-ADP-ribosyltransferase, catalyzes only the initial ADP-ribosylation of protein; in addition to protein, arginine residues or other compounds containing a guanidino group may serve as ADP-ribose acceptors (Moss & Vaughan, 1978; Moss et al., 1980). An enzyme with this substrate specificity has been purified to apparent homogeneity from turkey erythrocytes; it has a subunit molecular weight of ~28 000 (Moss et al., 1980). This NAD:arginine ADP-ribosyltransferase exists in an inactive aggregated form of high molecular weight and becomes activated upon dissociation. The conversion from the inactive to the active form is promoted by chaotropic salts or histones (Moss et al., 1981, 1982). The activity of the transferase thus appears to be sensitive to the local environment and quaternary structure. Since the enzyme appears to exist

† From the Laboratory of Cellular Metabolism and the Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205. *Received August 23, 1983.*