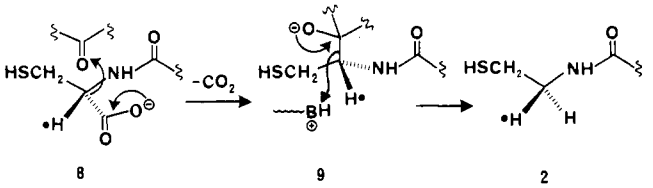
Scheme II: Hypothetical Reaction Mechanism for *P*PC Decarboxylase



then have to be highly conjugated) may be responsible for the reported (Scandurra et al., 1974) UV absorption of the purified enzyme, which shows a shoulder at 370–440 nm, decreased by addition of mercaptoethanol.

Alternatively, the carbonyl may act as a temporary acceptor of the presumed carbanion resulting from CO_2 elimination, with the formation of an intermediate such as **9** (Scheme II). Subsequent fragmentation of **9**, possibly with proton delivery by an active site lysine residue (whose presence is suggested on the basis of the observed inhibition by pyridoxal phosphate), would then yield the product **2**. The decarboxylation and protonation steps (Scheme II) might reasonably be formulated as proceeding with inversion of configuration, resulting in overall retention as observed. Obviously, further studies on this interesting enzyme will be required to confirm the presence and exact nature of the various reactive groupings in the active site.

ACKNOWLEDGMENTS

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L-Alanosine: A Noncooperative Substrate for *Escherichia coli* Aspartate Transcarbamylase†

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ABSTRACT: L-Alanosine, an antibiotic produced by *Streptomyces alanosinicus*, can be used by *Escherichia coli* aspartate transcarbamylase as a substrate instead of L-aspartate. The Michaelis constant of the catalytic subunit for this analogue is about 10 times higher than that for the physiological substrate, and the catalytic constant is about 30 times lower. The saturation curve of the native enzyme for L-alanosine indicates the lack of homotropic cooperative interactions between the catalytic sites for the utilization of this compound. It appears therefore that L-alanosine is unable to promote the allosteric transition. However, *N*-(phosphonoacetyl)-L-aspartate, a "bisubstrate analogue" of the physiological substrates, stimulates the reaction. This phenomenon is very similar to that reported by Foote and Lipscomb [Foote, J., & Lipscomb, W. N. (1981) *J. Biol. Chem. 256*, 11428–11433] concerning the reverse reaction using carbamylaspartate. The reaction is normally sensitive to the physiological effectors ATP and CTP. The significance of these results for the mechanism of the allosteric regulation is discussed.

Escherichia coli aspartate transcarbamylase (EC 2.1.3.2) catalyzes the first reaction of the pyrimidine pathway, that is, the carbamylation of the amino group of aspartate by carbamyl phosphate. This reaction is feedback-inhibited by CTP and stimulated by ATP. *E. coli* aspartate transcarbamylase (ATCase)[1] is made by the association of two

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[1] Abbreviations: ATCase, aspartate transcarbamylase; PALA, *N*-(phosphonoacetyl)-L-aspartate; T and R forms, tight and relaxed forms of the enzyme having low and high affinity, respectively, for the substrate aspartate; Tris, tris(hydroxymethyl)aminomethane; PEI–cellulose, poly(ethylenimine)–cellulose.

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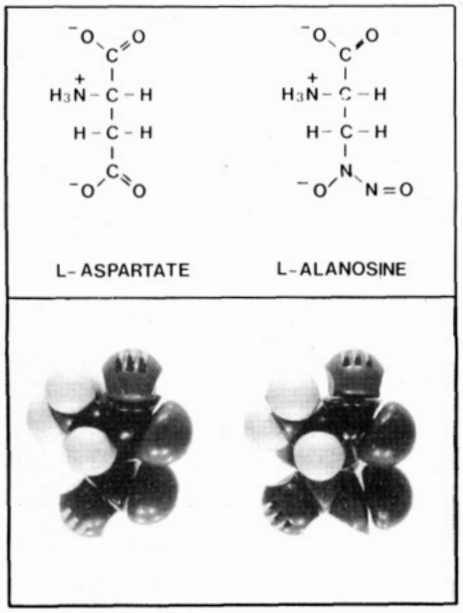

FIGURE 1: Chemical structure of L-alanosine as compared to that of L-aspartate.

catalytic trimers and three regulatory dimers. This enzyme is studied as a model for allosteric regulation, and its properties have been extensively reviewed (Jacobson & Stark, 1973a; Kantrowitz et al 1980a,b). ATCase shows homotropic cooperative interactions between the catalytic sites for aspartate binding. These interactions correspond to a transition from a T state (low affinity) to an R state (high affinity). This transition seems to be concerted in the sense that the maximal effect is observed before full saturation of the catalytic sites (Gerhart & Schachman, 1968; Hammes & Wu, 1971; Griffin et al., 1973; Kirschner & Schachman, 1973; Gibbons et al., 1976; Howlett et al., 1977; Johnson & Schachman, 1980). The two extremes states can be stabilized through modification of the protein–solvent interactions (Dreyfus et al., 1984). Several kinds of experimental data show that the ATCase molecule swells when going from the T to the R conformation (Gerhart & Schachman, 1968; Dubin & Cannell, 1975; Moody et al., 1979; Ladner et al., 1982). The heterotropic interactions that allow for the feedback inhibition of the enzyme activity by CTP and its stimulation by ATP are better explained through a "primary effect" of these nucleotides which is exerted locally site by site and a "secondary effect" on the T $\rightleftharpoons$ R equilibrium which is in fact promoted by the substrate aspartate (Thiry & Hervé, 1978; Tauc et al., 1982). This mechanism was recently confirmed by X-ray scattering experiments (Hervé et al., 1985). In view of the complexity of the regulatory interactions in this enzyme, experimental procedures that alter specifically some of these interactions have proved to be useful for studying their mechanisms. In several instances modified ATCases were obtained in which the homotropic cooperative interactions between the catalytic sites were selectively abolished (Kerbiriou & Hervé, 1972, 1973; Kerbiriou et al., 1977; Kantrowitz et al., 1977; Kantrowitz & Lipscomb, 1977; Landfear et al., 1978; Enns & Chan, 1978, 1979; Chan & Enns, 1979). Substrate analogues might also provide information by behaving differently from the physiological substrates in terms of catalysis and regulation.

L-Alanosine (Figure 1), an antibiotic produced by *Streptomyces alanosinicus* (Murthy et al., 1966), was found to inhibit the activity of ATCase from *Candida albicans* through competition with aspartate for the catalytic site (Gale et al., 1968). Furthermore, it has been reported that this compound can be carbamylated by mouse spleen ATCase (Jayaram & Cooney, 1979).

In this work, the influence of L-alanosine on the catalytic activity of *E. coli* ATCase was investigated. Indeed this compound can be used as a substrate instead of aspartate, but it appears to be unable to promote the allosteric transition.

Materials and Methods

Chemicals. ATP (sodium salt) and CTP (sodium salt) were purchased from Sigma Chemical Co. (St Louis, MO), L-aspartate was from Fluka (Buchs, Switzerland), [^{14}C]carbamyl phosphate (dilithium salt; 10 mCi/mmol) and Aquasol-2 were from New England Nuclear (Boston, MA), and formic acid and ninhydrin were from Merck (Darmstadt, West Germany). Other chemicals were purchased from Prolabo (Paris, France); *N*-(phosphonoacetyl)-L-aspartate (PALA) was a generous gift from Drs. L. Kedda and V. Narayanan, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NIH, Silver Spring, MD; L-alanosine was generously provided by Drs. D. Cooney, R. Davis, and V. Narayanan, National Cancer Institute, NIH, Bethesda, MD.

Enzyme Preparation. E. coli ATCase was prepared, and dissociated into catalytic and regulatory subunits, according to Gerhart & Holoubek (1967).

Enzyme Assay. (i) Method Using [^{14}C]Carbamyl Phosphate. Aspartate transcarbamylase activity was determined under the following conditions: in a total volume of 300 μL, 15 μmol of Tris–acetate buffer (pH 8), 1.5 μmol of [^{14}C]-carbamyl phosphate (0.15 mCi/mmol), either L-aspartate or L-alanosine, and ATCase as indicated were incubated for 10 min at 37 °C. The reaction was stopped by adding 1 mL of 1 N acetic acid. An aliquot (1 mL) of this mixture was transferred to a glass scintillation vial and evaporated to dryness at 80 °C to eliminate the degradation products of unreacted carbamyl phosphate. The residue was redissolved in 4.5 mL of distilled water, and 8 mL of Aquasol-2 was added. Radioactivity was measured by using an Intertechnique SL32 scintillation counter.

(ii) Method Using [^{14}C]-L-Aspartate. ATCase activity was determined by the method of Porter et al. (1969) under the conditions previously described (Perbal & Hervé, 1972) but in the presence of 5 mM carbamyl phosphate and 2 mM [^{14}C]-L-aspartate (0.15 mCi/mmol).

Influence of Effectors ATP and CTP. The influence of the physiological allosteric effectors ATP and CTP on the catalytic activity of ATCase with either L-aspartate or L-alanosine was tested and calculated as previously described (Tauc et al., 1982).

pH Dependence of the Catalytic Activity in the Presence of L-Aspartate or L-Alanosine. The pH dependence of the catalytic activity with either L-aspartate or L-alanosine was determined under standard conditions, but in the presence of 50 mM cacodylate buffer (from pH 6.0 to 7.4) or 50 mM Tris–acetate buffer (from pH 7.4 to 10.0) as previously described (Kerbiriou & Hervé, 1972).

Chromatographic Analysis of Carbamyl-L-alanosine. The carbamylalanosine formed was analyzed by ascending chromatography on PEI–cellulose plates (Macherey-Nagel, Sil G, UV_{254}) in parallel with L-alanosine, carbamyl phosphate, L-aspartate, and carbamylaspartate using 0.6 N formic acid as solvent. The spots were located either by autoradiography or by ninhydrin reaction.

Results

Influence of L-Alanosine on the Activity of the Catalytic Subunit of E. coli ATCase. Figure 2 shows that L-alanosine

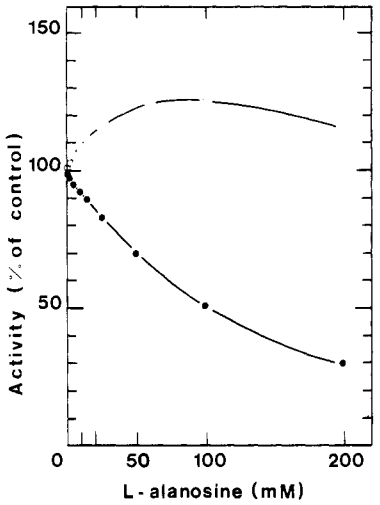

FIGURE 2: Influence of L-alanosine on the activity of the catalytic subunits and native ATCase. The activity of the catalytic subunits (0.12 μg) and of native ATCase (0.2 μg) was tested as described under Materials and Methods by using [^{14}C]-L-aspartate as substrate in the presence of increasing amounts of L-alanosine. Catalytic subunits (●); native ATCase (○).

inhibits the carbamylation of L-aspartate by the isolated catalytic subunit of *E. coli* ATCase. Under the conditions used (2 mM L-aspartate), 50% inhibition is observed in the presence of this analogue at a concentration of 100 mM. It was verified that L-alanosine acts as a competitive inhibitor toward L-aspartate.

L-Alanosine Is a Substrate for the Catalytic Subunit of ATCase. In order to determine whether L-alanosine can be used as a substrate, the isolated catalytic subunits of ATCase were incubated in the presence of this compound and [^{14}C]-carbamyl phosphate as described under Materials and Methods. It appears that indeed carbamylation of L-alanosine takes place. The product obtained was analyzed by chromatography on PEI–cellulose as described under Materials and Methods using L-aspartate, carbamyl phosphate, L-alanosine, and carbamylaspartate as controls. Carbamylalanosine migrated with a R_f value of 0.43 instead of 0.58 in the case of carbamylaspartate. It was verified that the rate of reaction varies linearly with the amount of enzyme and remains constant for at least 30 min under the conditions used.

The saturation curves for L-alanosine and L-aspartate are compared in Figure 3. Although the low affinity of the enzyme for L-alanosine renders the determination of the kinetic parameters difficult, the calculation made by using a computerized linear regression program indicates a K_m value of 180 mM (20 mM in the case of L-aspartate) and a maximum velocity of 0.028 mol min^{-1} (g of protein)$^{-1}$ as opposed to 0.8 mol min^{-1} (g of protein)$^{-1}$ in the case of L-aspartate.

Utilization of L-Alanosine by Native ATCase. L-Alanosine is also a substrate for native ATCase. However, it does not inhibit the carbamylation of L-aspartate in the same range of concentration as in the case of the catalytic subunits. Instead it provokes a slight activation whose maximum is centered at about 100 mM (Figure 2). It was interesting to determine the extent to which native ATCase exhibits regulatory interactions when this compound is used as substrate.

(*i*) *Saturation Curves.* The saturation curves for L-alanosine and L-aspartate of native ATCase are compared in Figure 4A. Although the low affinity of the enzyme for the substrate analogue did not allow saturation to be reached, it appears that, in contrast to what is observed with the natural substrate, the saturation curve for L-alanosine does not exhibit homotropic cooperative interactions in the utilization of this pseudosubstrate. This is confirmed by the corresponding double-reciprocal plots (Figure 4B). As already mentioned, the determination of the kinetic parameters is rendered difficult by the very low affinity of the catalytic site for L-alanosine. The apparent K_m estimated by using the computerized linear regression program is higher than 1 M.

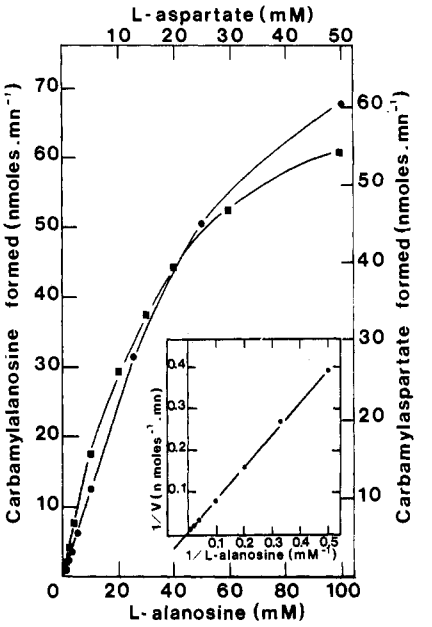

FIGURE 3: Saturation curves of isolated catalytic subunits of ATCase for L-alanosine and L-aspartate. Catalytic subunits of ATCase were incubated under the conditions described under Materials and Methods in the presence of [^{14}C]carbamyl phosphate and increasing amounts of either L-alanosine or L-aspartate, using 10 μg of enzyme in the presence of L-alanosine and 0.12 μg of enzyme in the presence of L-aspartate. L-Alanosine (●); L-aspartate (■). Insert: corresponding double-reciprocal plot in the case of L-alanosine.

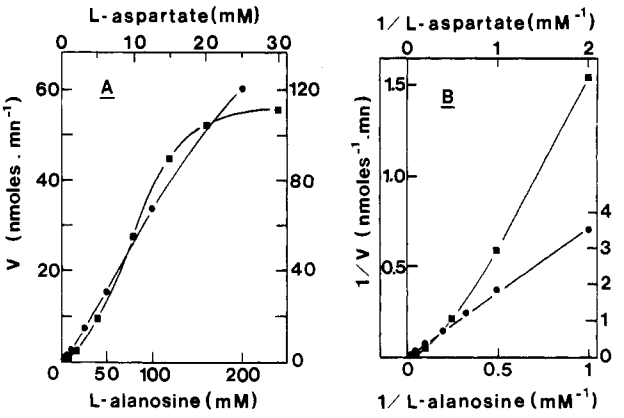

FIGURE 4: Utilization of L-alanosine as a substrate by native ATCase. Panel A: Saturation curves of native ATCase for L-alanosine and L-aspartate. ATCase (10 μg in the case of L-alanosine and 0.36 μg in the case of aspartate) was incubated under the conditions described under Materials and Methods in the presence of [^{14}C]carbamyl phosphate and increasing amounts of either L-alanosine (●) or L-aspartate (■). Panel B: Corresponding double-reciprocal plots.

(*ii*) *pH Dependence.* It has been known for a long time that the pH dependence of the reaction catalyzed by *E. coli* ATCase varies when the concentration of L-aspartate increases (Gerhart & Pardee, 1964; Kerbiriou & Hervé, 1972), and it has been shown that this phenomenon is absolutely correlated with the existence of the homotropic cooperative interactions between the catalytic sites (Thiry & Hervé, 1978). In the presence of low concentrations of L-aspartate, a condition under

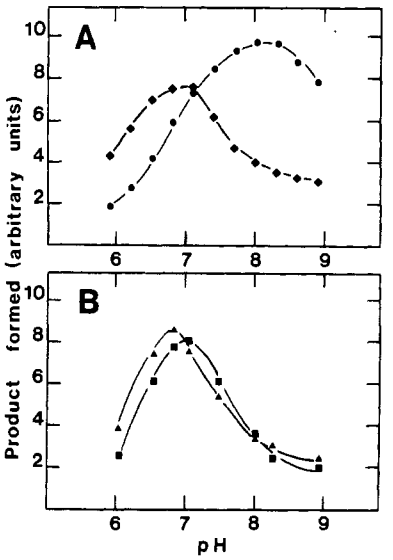

FIGURE 5: pH dependence of the enzymatic reaction using L-alanosine or L-aspartate. ATCase activity was determined at varying pHs as described under Materials and Methods. Panel A: pH dependence of the reaction in the presence of L-aspartate. One unit corresponds to 20 nmol when the incubation was performed in the presence of 1 mM L-aspartate (♦) and to 100 nmol when the reaction was performed in the presence of 20 mM L-aspartate (●). Samples of 0.36 μg of ATCase were used. Panel B: pH dependence of the reaction in the presence of L-alanosine. One unit corresponds to 1.25 nmol when the incubation was performed in the presence of 2 mM L-alanosine (▲) and to 50 nmol when the reaction was performed in the presence of 100 mM L-alanosine (■). Samples of 10 μg of ATCase were used.

which ATCase is essentially all in the T form, its optimum pH is 6.8 whereas in the presence of high concentrations of L-aspartate, a condition under which ATCase is in the R form, its optimum pH is 8.2. In contrast to native ATCase, both the isolated catalytic subunits and the modified enzymes in which the homotropic cooperative interactions have been selectively abolished and which are "frozen" in the R state exhibit an optimum pH of 8.2 whatever the concentration of L-aspartate (Kerbiriou & Hervé, 1972).

Since the pH dependence and its variation can provide information about the homotropic cooperative interactions between the catalytic sites, this parameter was examined here in the case of the carbamylation of L-alanosine catalyzed by the native enzyme. The results obtained are shown in Figure 5. In contrast with the fact that the optimum pH of ATCase varies from 7 to 8.2 when the concentration of L-aspartate varies from 1 to 20 mM, only a minor variation of the pH dependence is observed when the concentration of L-alanosine increases from 2 to 100 mM. Under these conditions, the optimum pH remains between 6.8 and 7.0, in accordance with the apparent absence of allosteric transition in this case.

(*iii*) *Heterotropic Interactions.* The influence of CTP and ATP, the physiological effectors of the enzyme, on the carbamylation reaction of L-alanosine was investigated under the usual conditions as indicated under Materials and Methods. Figure 6 shows that the reaction using the analogue is sensitive to the presence of the effectors, to about the same extent as the carbamylation of L-aspartate.

(*iv*) *Influence of PALA.* It has been previously shown that at low concentration, PALA, an analogue that combines the binding features of both substrates of ATCase (Collins & Stark, 1971; Jacobson & Stark, 1973b), stimulates the activity of ATCase by promoting the allosteric transition (Collins & Stark, 1971; Jacobson & Stark, 1975; Kantrowitz et al., 1977; Kerbiriou et al., 1977). Such an effect has been looked for in the case of the reaction using L-alanosine.

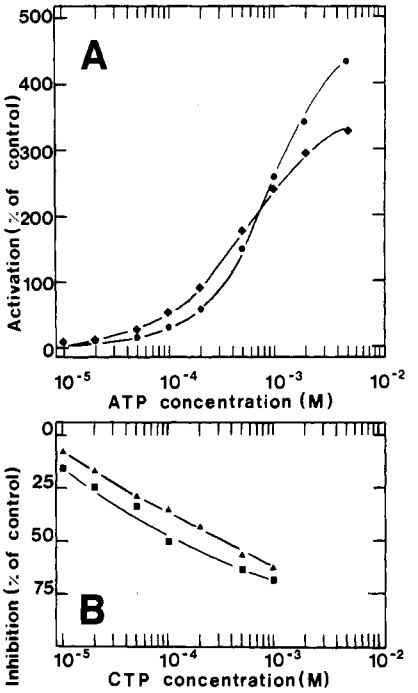

FIGURE 6: Influence of effectors ATP and CTP on the reaction catalyzed by ATCase in the presence of L-aspartate or L-alanosine. The influence of ATP and CTP on the reaction catalyzed by ATCase in the presence of L-alanosine (10 mM) or L-aspartate (1 mM) was determined as indicated under Materials and Methods, with 10 μg of ATCase in the case of L-alanosine and 1 μg in the case of aspartate. Panel A: Stimulation by ATP of the carbamylation of L-alanosine (●) and L-aspartate (♦). Panel B: Feedback inhibition by CTP of the carbamylation of L-alanosine (■) and L-aspartate (▲).

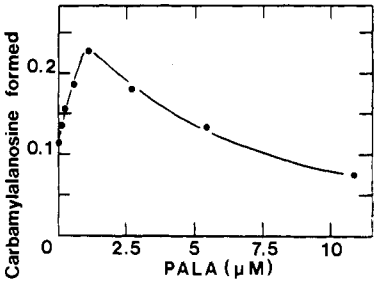

FIGURE 7: Influence of PALA on the enzymatic carbamylation of L-alanosine by ATCase. The enzymatic carbamylation of L-alanosine (6 mM) by ATCase (0.36 μg) was tested as indicated under Materials and Methods with [^{14}C]carbamyl phosphate, in the presence of increasing concentrations of PALA. Carbamylalanosine formed is expressed as nmoles per minute.

In Figure 7 it can be seen that indeed PALA stimulates the activity of ATCase at low L-alanosine concentration, indicating that the R conformation of the enzyme has a higher affinity for L-alanosine than the T conformation. This is in apparent contradiction to the lack of homotropic cooperative interactions between the catalytic sites for the utilization of this substrate analogue. This discrepancy will be discussed later.

DISCUSSION

It appears that L-alanosine can be used as a substrate by *E. coli* ATCase and its catalytic subunits, although with a lower affinity than the physiological substrate, L-aspartate. Surprisingly, ATCase uses L-alanosine in a noncooperative way.

A hyperbolic saturation curve for L-alanosine could be observed if the T and R forms would have the same or very close affinities for this substrate analogue. This possibility is excluded by the fact that the "bisubstrate analogue" PALA is

as effective in stimulating the utilization of L-alanosine as it is in the case of L-aspartate by promoting the allosteric transition toward the R conformation.

The fact that PALA stimulates the utilization of L-alanosine to the same extent as that of L-aspartate indicates that the T and R extreme conformations of the enzyme differ in their affinity for L-alanosine. This is in accordance with the fact that the isolated catalytic subunits have a much higher affinity for L-alanosine than the native enzyme as in the case of L-aspartate. In spite of the different affinities of the T and R forms for L-alanosine, this substrate analogue appears to be unable to promote the allosteric transition. Interestingly, the same conclusion has been reached by Foote et al. (1985) using L-cysteinesulfinate, another aspartate analogue that also behaves as a poor substrate for ATCase. In addition, these phenomena are strikingly similar to what has been reported by Foote & Lipscomb (1981) in the case of carbamylaspartate. These authors demonstrated that although PALA strongly stimulates the rate of the reverse reaction in which carbamylaspartate is the substrate, in the absence of this bisubstrate analogue the reverse reaction is exclusively catalyzed by the T form in a noncooperative way, carbamylaspartate being unable to induce the allosteric transition.

Taken together, these results indicate that the homotropic cooperative interactions in ATCase cannot be explained in terms of a simple two-state thermodynamic equilibrium which would be shifted by an exclusive binding to the R conformation. This interpretation is consistent with the shift of pH dependence of the reaction that accompanies the allosteric transition (Gerhart & Pardee, 1964; Kerbirious & Hervé, 1972; Thiry & Hervé, 1978). The T and R forms of the enzyme differ in their optimum pH for catalysis (pH 6.8 and 8.2, respectively). This variation of pH dependence indicates that, in the presence of low concentrations of L-aspartate, not only is the T form predominant but this form participates predominantly in the catalysis. However, the transition seems to be in some way concerted in the sense that it is complete before full saturation of the catalytic sites by the substrates (Gerhart & Schachman, 1968; Hammes & Wu, 1971; Griffin et al., 1973; Kirschner & Schachman, 1973; Gibbons et al., 1976; Howlett et al., 1977; Johnson & Schachman, 1980).

It must be remarked that the very low affinity of ATCase for L-alanosine did not allow the saturation curve for this pseudosubstrate to be entirely covered. It could be thus argued that this limited occupation of the catalytic sites might be responsible for the apparent lack of cooperativity. However, several observations suggest that this is not the case. First, the higher concentrations of L-alanosine used are superior to those that provoke the slight activation of the enzyme activity. In addition, it can be seen that in the case of L-aspartate the saturation curve is concave upward even for concentrations that are inferior to one-tenth of the $S_{0.5}$. Furthermore, in a double-reciprocal plot of such a sigmoidal saturation curve the values corresponding to the lower concentrations used extrapolate to largely negative estimates of K_m (Figure 4B). Such is not the case for the L-alanosine saturation curve.

Although L-alanosine appears to be unable to promote the allosteric transition toward the R conformation for which it has a higher affinity, its progressive binding to the catalytic sites might produce a very limited structural change in the direction of this conformation. This is suggested by both its light stimulatory effect on the aspartate reaction and the very discrete change in the pH dependence of the L-alanosine reaction when the concentration of this pseudosubstrate is raised from 2 to 100 mM. It is interesting to consider that this very small variation in subunit interactions promoted by L-alanosine binding to the catalytic site (compared to the effect of aspartate binding) should be related to the high value of the dissociation constant for L-alanosine. Such a free energy coupling between ligand binding and subunit association has been extensively discussed by Weber (1975, 1984).

Considering the three-dimensional structure of L-alanosine in comparison with that of L-aspartate (Figure 1), it appears that it is the N–OH group of this compound that is at about the same distance from the α-carboxyl group as is the β-carboxyl group in L-aspartate. Ionization of this group has been demonstrated (Coronelli et al., 1966) and can explain the binding of L-alanosine to the catalytic site of ATCase.

The uncoupling of homotropic and heterotropic interactions in ATCase was obtained in numerous instances through modifications of this enzyme. These modified forms of ATCase do not present anymore the homotropic cooperative interactions between the catalytic sites, but they are still sensitive to the effectors ATP and CTP (Kerbiriou & Hervé, 1972, 1973; Kantrowitz et al., 1977; Kerbiriou et al., 1977; Kantrowitz & Lipscomb, 1977; Landfear et al., 1978; Enns & Chan, 1978, 1979; Chan & Enns, 1979; Tauc et al., 1982). Interestingly, such an uncoupling is also observed here when normal ATCase is used with an analogue of substrate, bringing additional confirmation that these two types of interactions occur in ATCase through different molecular mechanisms (Thiry & Hervé, 1978; Tauc et al., 1982; Hervé et al., 1985).

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Registry No. ATCase, 9012-49-1; PALA, 51321-79-0; ATP, 56-65-5; CTP, 65-47-4; L-alanosine, 5854-93-3; L-aspartic acid, 56-84-8.

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Active-Site Modification of Mammalian Pyruvate Dehydrogenase by Pyridoxal 5′-Phosphate†

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ABSTRACT: The pyruvate dehydrogenase multienzyme complex from bovine kidney and heart is inactivated by treatment with pyridoxal 5′-phosphate and sodium cyanide or sodium borohydride. The site of this inhibition is the pyruvate dehydrogenase (E_1) component of the complex. Inactivation of E_1 by the pyridoxal phosphate–cyanide treatment was prevented by thiamin pyrophosphate. Equilibrium binding studies showed that E_1 contains two thiamin pyrophosphate binding sites per molecule ($\alpha_2\beta_2$) and that modification of E_1 increased the dissociation constant (K_d) for thiamin pyrophosphate about 50-fold. Incorporation of approximately 2.4 equiv of ^{14}CN per mole of E_1 tetramer in the presence of pyridoxal phosphate resulted in about a 90% loss of E_1 activity. Radioactivity was incorporated predominately into the E_1 α subunit. Radioactive N^6-pyridoxyllysine was identified in an acid hydrolysate of the E_1–pyridoxal phosphate complex that had been reduced with NaB^3H_4. The data are interpreted to indicate that in the presence of sodium cyanide or sodium borohydride, pyridoxal phosphate reacts with a lysine residue at or near the thiamin pyrophosphate binding site of E_1. This binding site is apparently located on the α subunit.

The mammalian pyruvate dehydrogenase complex is organized about a 60-subunit core, consisting of dihydrolipoamide acetyltransferase (E_2)[1] to which multiple copies of pyruvate dehydrogenase (E_1) and dihydrolipoamide dehydrogenase (E_3) are bound by noncovalent bonds (Reed, 1974). In solution, uncomplexed E_1 is a tetramer ($\alpha_2\beta_2$), and E_3 is a homodimer. E_1, E_2, and E_3 act in sequence according to eq 1–5 with eq 6 being the sum of these equations.

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[1] Abbreviations: PDC, pyruvate dehydrogenase complex; E_1, pyruvate dehydrogenase; E_2, dihydrolipoamide acetyltransferase; E_3, dihydrolipoamide dehydrogenase; TPP, thiamin pyrophosphate; $LipS_2$ and $Lip(SH)_2$, oxidized and reduced lipoic acid, respectively; NAD^+, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; CoA, coenzyme A; EDTA, ethylenediaminetetraacetic acid; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

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