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Allosteric Properties of the First Enzyme of the Histidine Operon

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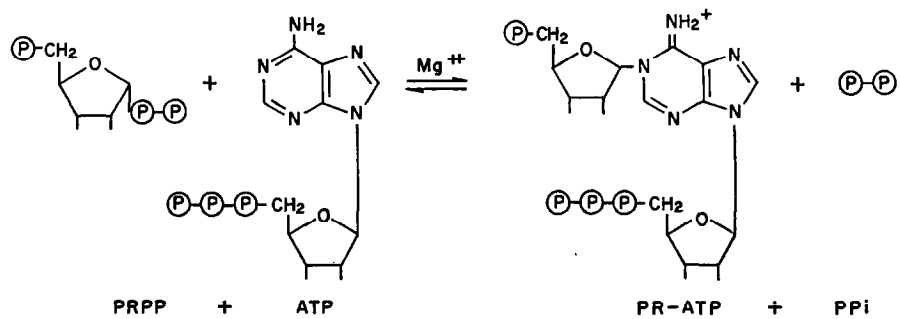
PR–ATP synthetase, the first enzyme of histidine biosynthesis of *Salmonella typhimurium* has been purified by an improved procedure which yields enzyme which migrates as a single band in both gel electrophoresis and electrofocusing experiments. When stored in glycerol solution at −15°C, PR–ATP synthetase remains fully active and sensitive to inhibition by histidine for extended time periods. The enzyme requires manganese or magnesium ions for activity and is activated by numerous monovalent cations. The pH optimum is 8 to 10 and is strongly dependent upon the buffer employed. The equilibrium constant for the reaction is 10^{-3}.

An assay for PRPP has been devised using PR–ATP synthetase. The enzyme showed negative cooperativity with ATP and Michaelis–Menten kinetics with histidine. A sulfhydryl group of the enzyme was found to be sensitive to conformational changes induced by ligands. Histidine accelerates the rate of reaction of this sulfhydryl group with iodoacetate, whereas ATP protects the sulfhydryl group from this reaction. The results can be interpreted as ligand induced sequential changes. ATP appears to induce a conformational change of one type which results in activation of the enzyme, protection of an SH group, and alteration in the interaction with neighboring subunits. Histidine on the other hand appears to cause a conformational change of another type which results in inhibition of the enzyme, exposure of an SH group, and no alteration in the interaction with neighboring subunits.

The first enzyme in the pathway of histidine biosynthesis in *Salmonella typhimurium*, *N*-1-(5′-phosphoribosyl)-adenosine triphosphate: pyrophosphate phosphoribosyl transferase (EC4.2.1c) was discovered by Ames and coworkers and shown to catalyze the transfer of the phosphoribosyl moiety from PRPP to ATP, as shown in Eq. (1) (*1*).



The product of the reaction, PR–ATP, is converted to histidine by a series of nine enzymatic steps (*2*). This enzyme, henceforth referred to as PR–ATP synthetase, is sensitive to feedback inhibition by histidine (*1*, *3*). The enzyme is of particular interest because it is typical of many control enzymes in being the first in a pathway, but contains

six sub-units instead of the more usual four (*1*, *4*). The mechanism of PR–ATP synthetase involves a covalent phosphoribosyl-enzyme intermediate (*5*). Because of the availability of a large number of mutants, altered forms of the enzyme could be made available for study. For these reasons a more specific study of its mechanism of action and regulatory properties appeared in order. This paper describes further studies on its purification, assay, and regulatory properties.

MATERIALS AND METHODS

Materials. Sephadex G-200 and DEAE Sephadex A-50 were purchased from Pharmacia. PRPP was the product of P-L Laboratories. Ultrapure urea was purchased from Mann. Yeast inorganic pyrophosphatase was obtained from Worthington. Hydroxyapatite (HPT) was obtained from Bio-Rad Laboratories. Iodoacetic acid was recrystallized three times from cyclohexene before use. All other chemicals were of the highest grade commercially available.

Enzyme assay. PR–ATP synthetase activity was assayed by the procedure of Voll *et al.* (*4*). The assay was performed at 25°C in 0.10 *M* Tris, 10 m*M* $MgCl_2$, 0.15 *M* KCl, 5 m*M* ATP, and 0.5 m*M* PRPP at pH 8.5. Yeast inorganic pyrophosphatase was added to drive the reaction to completion by hydrolyzing pyrophosphate.

Protein determination. Protein concentration was determined by the method of Lowry *et al.* (*6*) using bovine serum albumin (Armour Pharmaceutical Company) as standard. This standard gives higher values for protein than an insulin standard by a factor of 1.23.

A synthetic boundary experiment was performed to determine the extinction coefficient of the enzyme. One optical density unit at 280 mμ in standard buffer corresponds to a protein concentration of 1.59 mg/ml.

Electrophoresis techniques. Polyacrylamide gel electrophoresis at pH 8.5 was done essentially as described by Davis (*7*) in the presence and absence of 6 *M* urea.

Electrofocusing experiments were performed on an analytical scale using polyacrylamide gels for support in the presence and absence of 6 *M* urea (*8*). The preparative electrofocusing procedure employed was that described by Haglund (*9*).

Electrophoresis was performed on Cellogel strips (cellulose acetate) obtained from Colab Labs, Inc., Chicago, Ill. A colab electrophoresis apparatus No. 11-1880A Mark III was used. Electrophoresis was performed in Tris–borate-EDTA buffer, pH 8.6, at 250 v for 3 hr at 25°C (*10*). The protein was stained with Procion Brilliant Blue RS.

The subunit molecular weight of PR–ATP synthetase was estimated by gel electrophoresis in sodium dodecyl sulfate (SDS) using the procedure of Weber and Osborn (*11*).

Growth of bacteria. A histidine requiring mutant (hisE11) of *S. typhimurium*, LT-2 (kindly supplied by Dr. Bruce Ames) was grown under highly derepressing conditions for the enzymes of the histidine operon to maximize th eintracellular concentration of PR–ATP synthetase (*12*). The highest specific activity of PR–ATP synthetase was obtained from cells grown on the minimal medium of Vogel and Bonner (*13*) supplemented with 0.01 m*M* L-histidine, 0.071 m*M* L-histidinol and 0.4 m*M* adenine. Cells were grown with aeration at 37°C in either 15-liter carboys or in the 12-liter fermentation units of the New Brunswick fermentor. Alternatively, high specific activities were obtained in cells grown as described above without histidinol when 16–24 hr were allowed to pass after log phase had ended. About 0.5 g. of cells (wet weight) were obtained per liter. Cells were stored frozen at −15°C until used.

Standard buffer. The standard buffer consisted of 0.01 *M* Tris, 0.10 *M* NaCl, 0.4 m*M* histidine, 0.5 m*M* EDTA, and 1.0 m*M* dithiothreitol, pH 7.5.

Storage of enzyme. Since storage of purified PR–ATP synthetase in standard buffer at 4°C leads to loss of activity, a procedure to stabilize the enzyme was developed. It consists of the addition of 50% glycerol by volume to the enzyme in standard buffer at a protein concentration of 2–3 mg/ml, and storage at −15°C. The enzyme was stable for over 6 months under these conditions. Under some circumstances losses of activity were noted for unknown reasons.

Crystallization of PR–ATP synthetase. The enzyme was prepared by a modification of the procedure of Voll *et al.* (*4*). The modifications include the addition of a hydroxyapatite chromatographic step and the heat step of Whitfield (*23*) together with other minor changes. The enzyme was crystallized by the procedure of Jakoby (*14*). About 10 mg of enzyme were precipitated with 65% saturated ammonium sulfate in standard buffer at 0°C and the precipitate was successively back extracted after centrifugation with 1 ml of buffer at the following ammonium sulfate saturations: 60, 56, 52, 48, 44, 40, and 35%. The tubes were allowed to stand at room temperature for 24 hr. Small needle shaped crystals were observed using a light microscope at 300× magnification in all but the 60 and 56% saturation tubes. The best crystals were obtained from the 44% saturation tube. Long crystals (10–30 × 2 microns) were observed. These crystals constituted 25–50% of the material in view. Amorphous material was often found in association with the crystals.

RESULTS

Molecular weight of native enzyme. The molecular weight of the purified enzyme was determined by the meniscus depletion method of Yphantis (*15*). Enzyme at three different concentrations was sedimented to equilibrium in standard buffer at 12,590 rpm for 96 hr at 9°C. Plots of $\log C$ against X^2 were linear. For the calculation of molecular weight a $\bar{v}$ of 0.747 ml/g was used (*4*). The weight average molecular weights for the three solutions (initial protein concentrations of 1.67, 1.34, and 1.02 mg/ml) were 217 200, 216 700, and 219 300, respectively. These values are in good agreement with those previously reported (*4*).

A molecular weight of 222 000 ± 10 000 has been determined by chromatographing PR–ATP synthetase with proteins of known molecular weight on G-200 Sephadex by the procedure of Leach and O'Shea (*16*). A plot of the elution volume versus the logarithm of the molecular weight of BSA, egg albumin, and γ-globulin gave a straight line and the position of PR–ATP synthetase indicated a molecular weight near 222 000 in good agreement with the sedimentation equilibrium run.

Examination of purified PR–ATP synthetase at a concentration of 1 mg/ml in the ultracentrifuge using uv scanning optics revealed only one sedimenting peak which had an $S_{20°,w}$ of 8.32 ± 0.10. These values are lower than the values of 8.6–8.8 previously reported (*4*).

Molecular weight of subunit. SDS gel electrophoresis (Fig. 1) indicated a molecular weight for the subunit of 31 000. This finding is compatible with about 6 subunits per native enzyme molecule in agreement with the studies of Voll *et al.* (*4*).

Electrophoresis. Examination of purified PR–ATP synthetase on polyacrylamide gel electrophoresis by the method of Davis (*7*) revealed one major band with several minor components. The minor components account for no more than 3–5% of the total protein. Polyacrylamide gel electrophoresis run in 6 *M* urea revealed one major band.

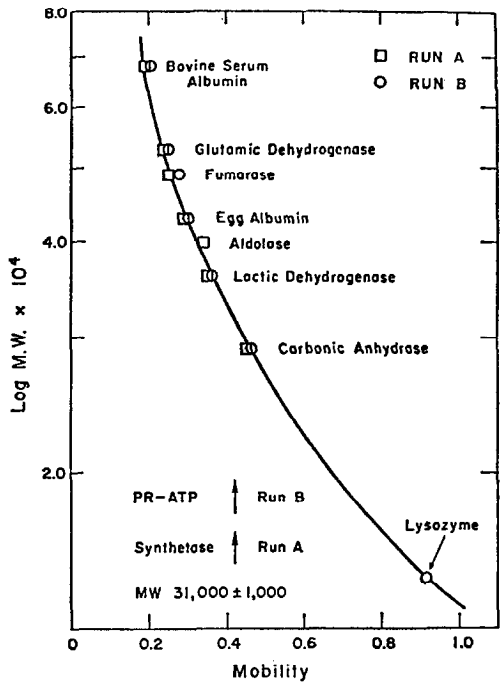


FIG. 1. Estimation of subunit molecular weight of PR–ATP synthetase by SDS gel electrophoresis. The mobility of several proteins of known molecular weight (listed in reference) and of PR–ATP synthetase were determined in SDS in 10% gels by the procedure of Weber and Osborn (*11*). Two independent determinations of the mobilities are shown, Run A and Run B

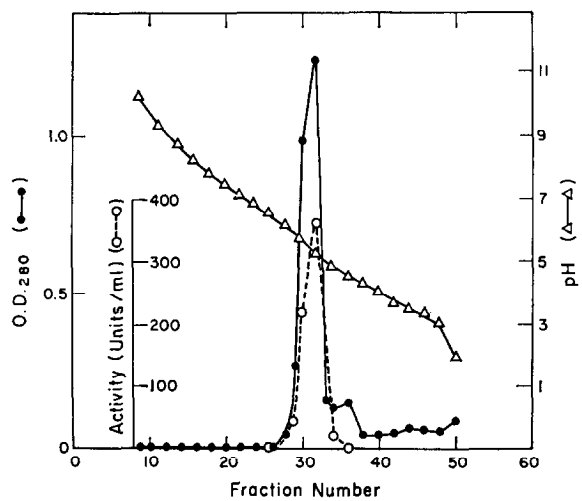


FIG. 2. Electrofocusing of PR–ATP synthetase. Four mg of PR–ATP synthetase (spec. act 750 U/o.d. 280) were focused using the LKB 8100 ampholine 110-ml electrofocusing apparatus. The pH 3–10 ampholite was employed at a concentration of 0.67%. The material was focused at 600 v for 18 hr at 9°C. A light precipitate appeared in Tubes 30–32.

TABLE 1

EQUILIBRIUM CONSTANT FOR THE REACTION CATALYZED BY PR–ATP SYNTHETASE[a]

$$PRPP + ATP \rightleftharpoons PR\text{–}ATP + PP_i$$

Initial concentrations				Observed	Calculated final concentrations				
PRPP mM	ATP mM	PR–ATP mM	PP_i mM	change in o.d. 290 mμ	PRPP mM	ATP mM	PR–ATP mM	PP_i mM	Equilibrium constant[b] $K = (PR\text{–}ATP)(PP_i)/(ATP)(PRPP) \times 10^4$
1.792	2.000	0	0	.214	1.731	1.939	0.612	.0612	11.2
0.896	1.000	0	0	.127	0.860	0.964	.0362	.0362	15.8
0.896	2.000	0	0	.168	0.848	1.952	.0428	.0428	13.9
1.792	1.000	0	0	.150	1.749	0.957	.0428	.0428	10.9
1.792	3.000	0	0	.171	1.730	2.938	.0620	.0620	7.6
							Average		11.9×10^{-4}

[a] Calculated from extinction difference (3.5×10^3) between PR–ATP and ATP at 290 mμ, pH 8.5 (1, 17).
[b] All measurements were made at 25°C.

Similarly, cellogel electrophoresis revealed one major protein band with one faint slower moving component.

Electrofocusing experiments have been performed on PR–ATP synthetase on both an analytical and preparative scale. When purified PR–ATP synthetase was focused using ampholites with a pH range of 3–10, one major band was observed with several faint bands. The addition of 6 *M* urea to such a focusing experiment revealed only one major protein band using ampholites with a range of 3–10 and 5–7. The results of a preparative electrofocusing experiment on the native enzyme, giving an isoelectric point for the enzyme of 6, are shown in Fig. 2. The major protein peak coincided with the enzymatic activity.

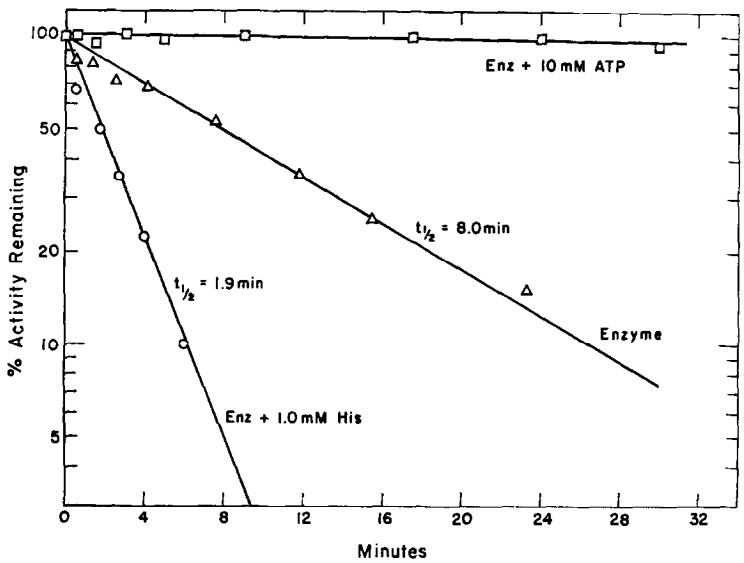


FIG. 3. Iodoacetate inhibition of PR–ATP synthetase. Purified PR–ATP synthetase was dialyzed against standard pH 8.5 buffer without histidine and dithiothreitol. The enzyme solution was diluted so that the final buffer concentration was 0.10 *M* Tris, 10 m*M* $MgCl_2$, 0.15 *M* KCl, and 20 m*M* iodoacetate at pH 8.5.

All experiments were performed at 25°C. At the times indicated aliquots were removed and assayed using the normal enzymatic assay.

Equilibrium constant for the reaction catalyzed by PR–ATP synthetase. The equilibrium constant for the reaction shown in Eq. (1) was estimated by spectroscopically measuring the production of PR–ATP at 290 mμ at pH 8.5 (*1*, *17*). The magnitude of the equilibrium constant is about 10^{-3} as shown in Table 1. The equilibrium was measured starting from PRPP and ATP. Perturbation of an established equilibrium by the addition of pyrophosphate caused a new equilibrium concentration of reactants and products to be established and the calculated equilibrium constant from these data was of the same order of magnitude.

Iodoacetate inactivation studies. The inactivation of PR–ATP synthetase by iodoacetate was studied to learn the effect of ligands upon the conformation of the enzyme. The results of three such experiments are shown in Fig. 3, where a semilog plot of the percent activity remaining versus time is shown for enzyme alone, enzyme plus 10 m*M* ATP, and enzyme plus 1 m*M* histidine. Histidine accelerated the first order inactivation rate in comparison to enzyme without effectors while ATP provided nearly 100%

protection. From analogous semilog plots, the half-lives for inactivation by iodoacetate in the presence of other substrates and analogues were determined and summarized in Table 2. Previous studies have demonstrated the strict specificity for histidine of the

TABLE 2

INACTIVATION PR–ATP SYNTHETASE BY IODOACETATE IN THE PRESENCE OF EFFECTORS[a]

Effector	Effector Concentration mM	$T_{1/2}$[b] (Min)
None		8.6
Histidine	1	1.9
	0.6	4.2
ATP	10	>100
ADP	10	96
AMP	10	21.6
Adenine	10	7.2
PRPP	1	>100
Ribose 5-phosphate	10	20.4
Histidine + ATP	1 (Histidine)	
	10 (ATP)	14.2
Histidine + PRPP	0.6 (Histidine)	
	1 (PRPP)	45.9

[a] The half-life for inactivation of PR–ATP synthetase by iodoacetate was determined under the conditions described in Fig. 6 in the presence of various effectors.

[b] Rates were normalized to one enzyme concentration.

regulatory site of this enzyme (*1–3*). Other amino acids do not affect the SH reactivity. AMP, ADP, and ATP protected against inactivation whereas adenine was without effect. PRPP and ribose-5-phosphate also protected against inactivation. When ATP

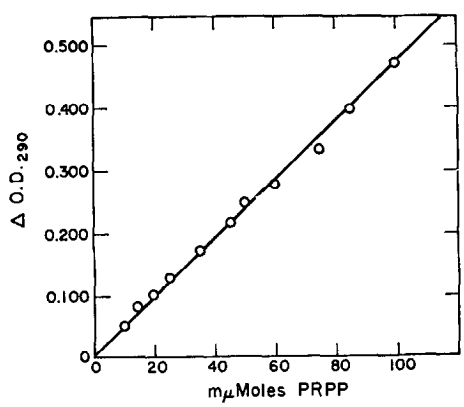


FIG. 4. PRPP assay. The optical density change at 290 mμ was determined using a Gilford recording spectrometer, when small aliquots of a standard PRPP solution were added to cuvettes containing PR–ATP synthetase, yeast inorganic pyrophosphotase, 0.10 *M* Tris, 10 m*M* $MgCl_2$, 0.15 *M* KCl, and 5.0 m*M* ATP at pH 8.5 in a final volume of 0.30 ml at 25°C.

and histidine were tested at the same time, one being a protector and the other an accelerator of inactivation, an intermediate rate of inactivation was observed. This was also true for the pair of PRPP and histidine. Definite differences in the rate of inactivation by iodoacetate in the presence of effectors suggest that different ligand induced conformational states for PR–ATP synthetase exist.

Assay for PRPP. The PR–ATP synthetase assay provides a sensitive assay procedure for PRPP. A direct linear relationship was found to exist between the mμmoles of PRPP added and the change in optical density at 290 mμ. Care must be taken in controlling pH as the absorption is sensitive to changes in pH. A standard curve is shown in Fig. 4 which utilized commercial PRPP as a standard. For absolute quantitation other

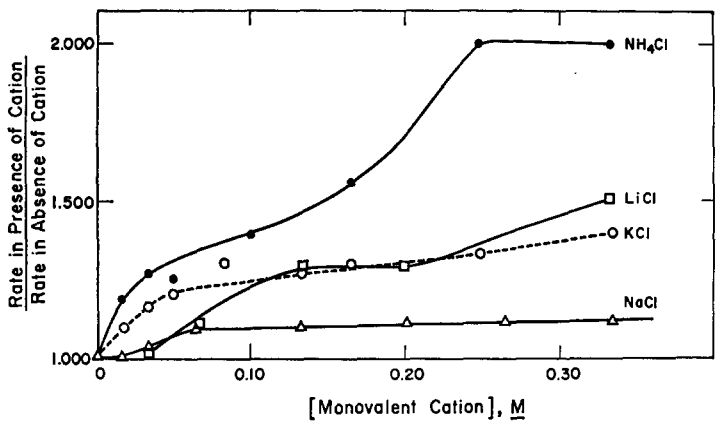


FIG. 5. Effect of monovalent cations on PR–ATP synthetase activity. PR–ATP synthetase was assayed at various concentrations of monovalent cations under the following conditions: 0.10 *M* Tris, 20 m*M* $MgCl_2$, 5.0 m*M* ATP, and 0.5 m*M* PRPP at pH 8.5 and at 25°C. All assays contained about 11 m*M* sodium ion because the sodium salts of ATP and PRPP were used. The monovalent cation concentration plotted was that added above this background level. Yeast inorganic pyrophosphatase was included in each reaction mixture.

independent analysis for the PRPP standard would be required. However, this observation appears to form the basis for a good assay for PRPP.

Divalent metal cation requirements. A divalent cation is required by PR–ATP synthetase for activity. The standard assay employs magnesium (4). The specificity of this divalent metal cation requirement was tested using standard assay conditions but substituting Zn^{2+}, Cu^{2+}, Ni^{2+}, or Mn^{2+} for magnesium at a concentration of 10 m*M*. Of the metal cations tested, only manganese would substitute for magnesium. The other cations gave rates of less than 1% of the rate in the presence of magnesium.

Monovalent cation activation. Activation of PR–ATP synthetase activity by monovalent cations has been observed previously (*4*). The effect of several monovalent cations upon the activity of PR–ATP synthetase is shown in Fig. 5. Ammonium ion, which had the greatest activating power, was followed by lithium and potassium ion. Sodium ion showed the smallest effect. Addition of a second cation at a saturating concentration of another cation resulted in additional activation, suggesting independent monovalent cation sites or an interaction between sites binding different cations.

pH Activity profile. The effect of pH (and buffer) upon PR–ATP synthetase activity is shown in Fig. 6. Since the extinction coefficient for PR–ATP is pH dependent, the observed rate at each pH was corrected for the change in the extinction coefficient of

PR–ATP using the data of Smith and Ames (*17*). The pH activity profile is complex, showing different maxima depending upon the buffer.

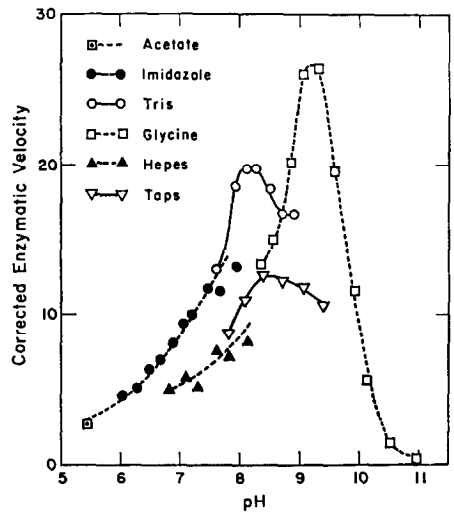
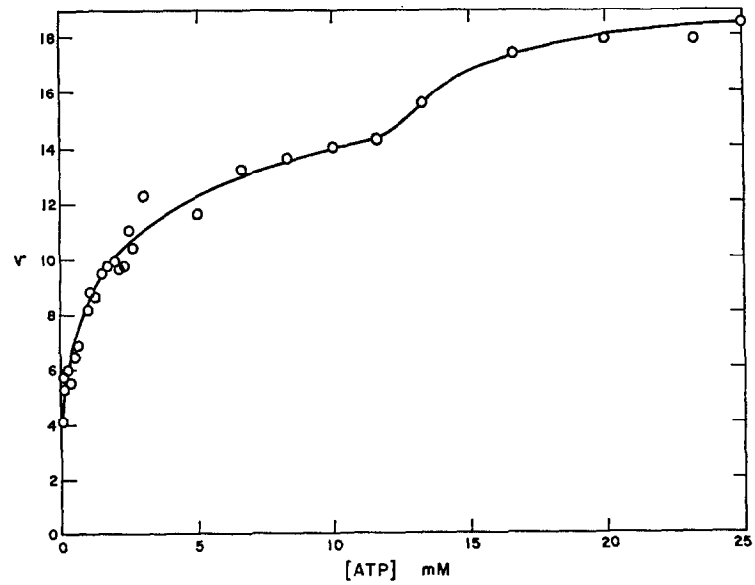


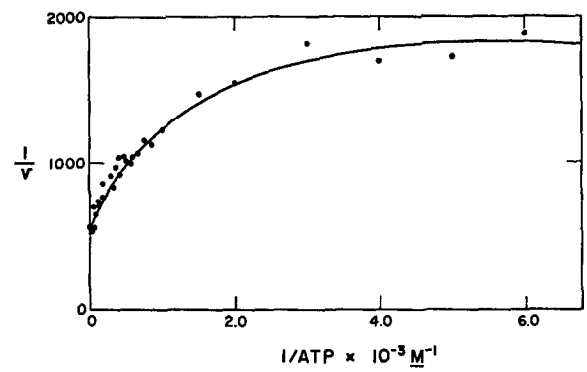
FIG. 6. pH activity profile for PR–ATP synthetase. Initial velocities for PR–ATP synthetase were measured by following the optical density change at 290 mμ under the following conditions: 1.0 *M* buffer, 16.4 m*M* $MgCl_2$, 5.0 m*M* ATP, 0.5 m*M* PRPP at 25°C and the pH indicated. Yeast inorganic pyrophosphatase was included in each reaction mixture. The observed rates were corrected as described in the text for the change in the extinction coefficient of PR–ATP with pH. (Hepes-*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, TAPS-tris(hydroxymethyl)methyl-aminopropane sulfonic acid).

ATP Kinetics. The ATP kinetics of PR–ATP synthetase are complex. A certain lack of reproducibility was noted, and the results must, therefore, be considered indicative rather than definitive. A direct plot of enzymatic velocity versus ATP concentration is shown in Fig. 7a, a double reciprocal plot for the same data is shown in Fig. 7b, and a Hill plot for the same data is shown in Fig. 7c. Hill numbers near one were calculated at both high and low ATP concentrations while a Hill number of about 0.5 was calculated at intermediate ATP concentrations. The cooperativity index, the ratio between 90 and 10% saturation, $S_{0.9}/S_{0.1}$, is equal to 81 for enzymes following Michalis–Menten kinetics, less than 81 for positively cooperative systems, and greater than 81 for negatively cooperative systems. The cooperativity index for PR–ATP synthetase was greater than 300 under these conditions. Such data are diagnostic of negative cooperativity (*18*, *19*). In this case such a conclusion is indicated but until the effects of monovalent cations and enzyme reproducibility are solved this must remain a working hypothesis.

PRPP Kinetics. PRPP appears to saturate the enzyme at concentrations lower than 3×10^{-5} *M* (Fig. 8). Further kinetic analysis was limited because of the low extinction coefficient for PR–ATP, i.e., 3.6×10^{-3} at pH 8.5 (*17*).

Histidine inhibition. The effect of histidine on the activity of the enzyme was determined as a function of histidine concentration and the results are shown in Fig. 9. The data seem to follow a Michaelis–Menten curve within experimental error giving an inhibition constant of 5×10^{-5} *M*. There is evidence however that, although Hill plots of 0.99 (as in this case) are obtained under these conditions, slopes differing from 1 are found under other situations.





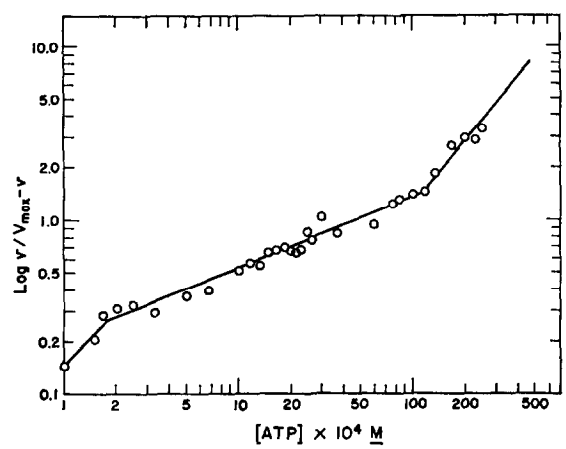


FIG. 7. ATP kinetics. Initial velocities of purified PR–ATP synthetase were measured at 25°C and pH 8.5 in 0.10 *M* Tris, 20 m*M* $MgCl_2$, 0.15 *M* KCl, and 0.5 m*M* PRPP which contained yeast inorganic pyrophosphatase. (a) Plot of initial velocity versus ATP concentration, (b) double reciprocal plot, and (c) Hill plot.

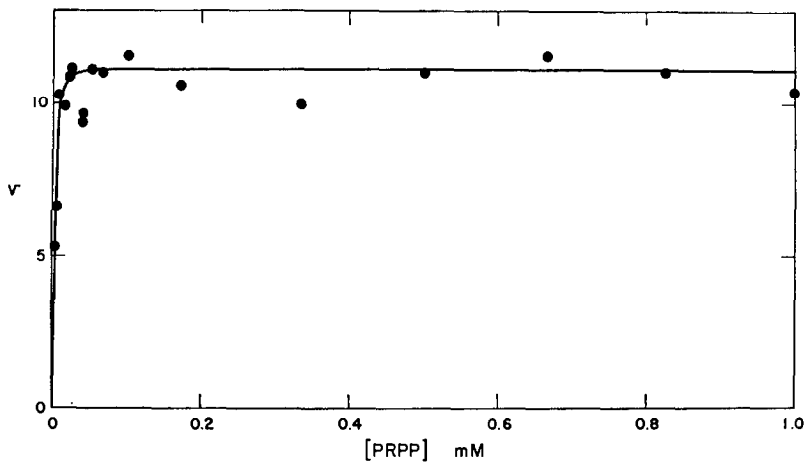


FIG. 8. PRPP kinetics. Initial velocities of purified PR–ATP synthetase were measured at 25°C and pH 8.5 in 0.10 *M* Tris, 5.0 m*M* ATP, 10 m*M* $MgCl_2$, and 0.15 *M* KCl. All assays contained yeast inorganic pyrophosphatase.

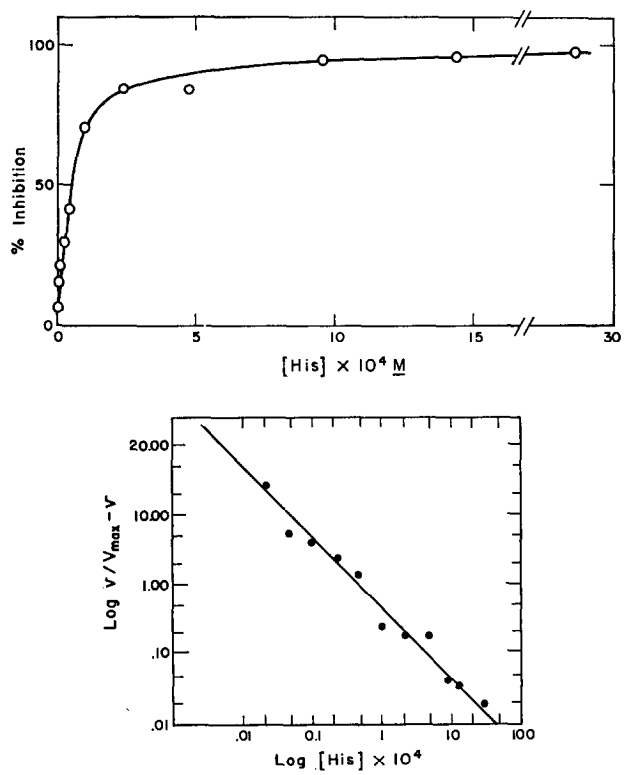


FIG. 9. Inhibition of PR–ATP synthetase by histidine. Initial velocities for PR–ATP synthetase under standard assay conditions (see methods) were measured prior to the addition of a small aliquot of L-histidine. Then an inhibited rate was measured. (a) The percent inhibition versus histidine concentration, and (b) Hill plot.

Another example of the kinetic complexity of PR–ATP synthetase is shown in Fig. 10 where a schematic representation of a typical assay and extent of histidine inhibition is shown. A lag phase was usually seen before the assay became linear. The rates reported in this paper come from this linear portion of the assay. The lag phase indicates a slow conformational change similar to the slow conformational change reported by Hatfield *et al.* for threonine deaminase (*20*). The addition of histidine immediately caused a sharp break in the rate, and the assay became nonlinear; but after several minutes a new linear rate was achieved. This transition from a linear assay to a nonlinear assay upon

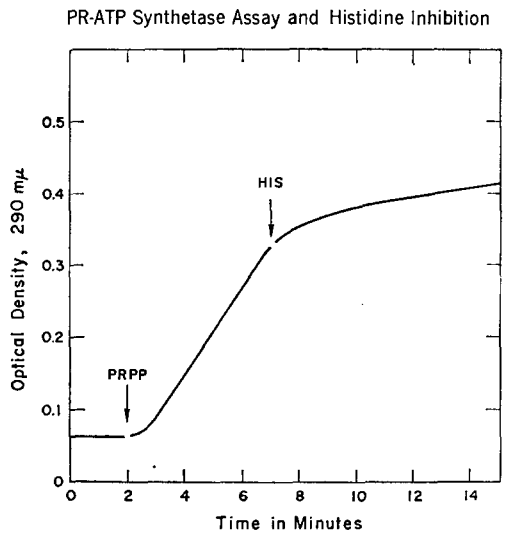


FIG. 10. PR–ATP synthetase assay. Schematic representation of a typical assay of PR–ATP synthetase as described under Methods showing a lag phase and the nonlinearity of assay upon addition of histidine (see text for details).

the addition of histidine strongly suggests that a slow histidine-dependent conformational change has been detected.

An apparent synergism between histidine and PR–ATP exists because PR–ATP must be present before histidine becomes inhibitory, i.e., addition of histidine prior to starting the reaction with PRPP leads to essentially the same initial rate as that found in the absence of histidine. The production of a small amount of product leads to inhibition and the rapid bending of the assay to an inhibited rate. Other experiments indicate that the extent of inhibition at a fixed histidine concentration is dependent upon the quantity of PR–ATP present. When histidine was added at high PR–ATP concentrations, a sharp break in the assay occurred which was not followed by a nonlinear region. Similar observations have been made on the PR–ATP synthetase from *Escherichia coli* (*21*).

Specificity. The specificity of the enzyme was investigated previously (*1, 3*) but a few additional features have been revealed. The use of the β-γ methlene analog of ATP, [ADPCP] (*22*), results in the formation of PR–ADPCP, indicating that ADPCP is an acceptable substrate for the enzyme. Also, an analogue of PRPP, PRPCP, has been prepared and was found to be a substrate for the enzyme.

The possibility that imidazole plus analine or imidazole plus glycine could substitute

for histidine was tested using high concentrations (100 mM) of these compounds. No inhibition was observed. This adds to the findings of Martin which indicated that this enzyme has a high specificity for histidine.

DISCUSSION

General properties. Further studies on the purification and properties of PR–ATP synthetase has resulted in an improved method of purification and observations concerning its physical and kinetic properties. Support for the six subunit nature of the enzyme and strong evidence that the subunits are identical have been obtained by the new electrofocusing and electrophoresis techniques. The improved method for the purification of the enzyme which has been devised provides enzyme of high specific activity. A storage procedure in glycerol allows enzyme to be kept for periods longer than 6 months.

The spectral change observed in the production of PR–ATP from PRPP and ATP is sufficient to provide a sensitive assay for PRPP.

Several factors were found to effect enzymatic activity. The enzyme has an absolute requirement for divalent cations. Magnesium and manganese could satisfy this requirment whereas several other divalent cations were ineffective. PR–ATP synthetase was activated by monovalent cations in a manner which suggests interacting or independent monovalent cation sites. The pH activity profile for the enzyme showed a pH optimum in the range of 8–10 which was dependent upon the buffer employed.

PR–ATP synthetase is highly specific for histidine and its substrates (*1*, *3*). The near absolute specificity for histidine has been supported by experiments in which mixtures of imidazole and glycine or imidazole and alanine were found not to inhibit the enzyme. However, the methylene analogs of ATP and PRPP were found to serve as substrates.

Kinetics and conformational changes. The ATP kinetics of this enzyme are complex. A double reciprocal plot or a Hill plot indicates that the enzyme is negative cooperative under these conditions. It appears to be Michaelis–Menten under other conditions (*3*, *23*). Since the enzyme is composed of identical subunits, the binding of ATP evidently can cause conformational changes which affect neighboring subunits in such a way that the binding affinity for subsequent ATP molecules is diminished. This can only occur if there is a sequential binding pattern for the ligand induced changes in individual subunits (*18*, *19*, *24*).

The histidine inhibition of the enzyme follows a Michaelis–Menten dependence and shows a Hill plot of slope 1. A Michaelis–Menten pattern can be obtained if there is no change in conformation, but in this case a histidine induced conformation change is indicated from both the regulatory effects and from the iodoacetate inactivation studies reported here. Histidine, therefore, appears to cause a conformational change which affects the turnover at the active site, but this conformational change does not extend to neighboring subunits under some conditions. Under other conditions cooperativity is observed (*23*, *25*). Michaelis–Menten binding is consistent with a ligand induced process (*24*) in which the subunit interaction terms K_{AB} and K_{BB} equal 1. Thus, a conformational change occurs on histidine binding which deactivates the active site within the same subunit but does not affect neighboring subunits.

The mode of histidine inhibition appears to involve an interaction between PR–ATP and histidine. Addition of histidine prior to substrates led to PR–ATP production at essentially an uninhibited rate; the greater the quantity of PR–ATP produced prior to the addition of a fixed amount of histidine, the greater the inhibition. The addition

of histidine apparently causes a slow time dependent conformational change of the enzyme, because at low PR–ATP concentrations nonlinear inhibited rates were seen, followed by a linear inhibited rate. Similar observations have been made on the PR–ATP synthetase isolated from *E. coli* (*21*). PR–ATP synthetase undergoes other slow time dependent conformational changes which are temperature and substrate dependent. These studies will be the subject of a separate communication.

Iodoacetate inactivation. PR–ATP synthetase has previously been shown to be inactivated by PCMB (*3*). The kinetics of inactivation of PR–ATP synthetase by iodoacetate are first order. The rate of inactivation apparently monitors the conformational state of the enzyme. Addition of ATP strongly protected the enzyme against inactivation by iodoacetate. ADP, AMP, and ribose-5-phosphate also protected against inactivation, whereas adenine was without effect. PRPP also protected against inactivation. On the other hand histidine accelerated the rate of inactivation. This indicates that histidine induces a conformational change of a different type than that induced by the substrates. A mixture of histidine and substrate (ATP) resulted in intermediate reactivity, as might be expected.

These inactivation studies, together with the kinetics, add an interesting chapter to the story of conformational changes in proteins. Conformational changes are indicated both by the observed negative cooperativity and by iodoacetate inactivation. Moreover, it can be seen that this is not an equilibrium between preexisting states, since the form of the protein induced by ATP which results in subunit interactions of the negatively cooperative type is quite different from the change induced by histidine which gives Michaelis–Menten kinetics. That the two induced conformational states are different is further indicated by the difference in SH reactivity, i.e., ATP causes a decreased rate of reaction with iodoacetate whereas histidine causes an increased rate of reaction. Allosteric control may occur by ligand induced changes which are localized between a regulatory site and an active site within one subunit. Cooperativity involves interaction between two similar sites. Cooperativity and control therefore are two manifestations of a similar process, the ligand induced change, but they are not necessarily connected. Some ligand induced changes may be manifest only in an individual subunit; others extend to neighboring subunits.

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