Characterization of Slowly Interconvertible States of Phosphoribosyladenosine Triphosphate Synthetase Dependent on Temperature, Substrates, and Histidine*

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(Received for publication, June 25, 1973)

SUMMARY

Phosphoribosyladenosine triphosphate (PR-ATP) synthetase from Salmonella typhimurium was found to undergo a slow temperature- and ligand-dependent activation. The kinetics of activation appeared first order with half lives up to 17 min depending on the conditions. Activation by the substrate ATP diminished the extent of subsequent temperature activation. The extent of activation appeared to be independent of pH and buffer concentration over limited ranges and was reversible. Two high temperature (25–37°C) states of PR-ATP synthetase having different inhibition responses toward histidine exist depending upon prior ligand action. Apparent patterns of histidine binding determined from kinetic data are similar to those determined from equilibrium data obtained by dialysis and protein fluorescence enhancement. The possible metabolic importance of the high temperature “hysteretic” behavior is discussed.

Substrate kinetics and histidine inhibition of PR-ATP synthetase (EC 2.4.2) have been studied previously with confusing results. Martin (1) and Whitfield (2) each found Michaelis-Menten kinetics for both ATP and PRPP indicative of a reactive ternary complex while Bell and Koshland (3) found negative cooperativity in the ATTP kinetics. Also, Bell and Koshland (4) have isolated a phosphoribosyl covalent enzyme intermediate, thus proving a double displacement mechanism which could operate in the absence of a ternary complex. Whitfield (2) and Martin (1) and Bell and Koshland (3) found histidine inhibition to be cooperative but incomplete while Martin (1) and Bell and Koshland (3) found it to be uncooperative and complete.

The recent studies described in the accompanying manuscripts indicate two factors which might help explain these discrepancies. In the first place the association-dissociation behavior is complex (5). Although nominally a hexamer of identical subunits, PR-ATP synthetase can be dissociated to a dimer at pH 10 and low ionic strength. Also, low temperature or high ionic strength leads to a more complex aggregation. In the second place, PR-ATP synthetase was found to contain a histidase impurity (7). Since histidase produces urocanic acid which absorbs in the ultraviolet spectral region, appreciable errors in the kinetic assays of PR-ATP synthetase occurred when high levels of histidine were present.

The production of a PR-ATP synthetase free of histidase activity and the understanding of the complex association-dissociation behavior of the enzyme has led us to re-examine some kinetic and equilibrium behavior of PR-ATP synthetase. During these studies a temperature- and ligand-dependent activation of the enzyme has been observed. A coherent picture emerges which appears to explain the previous inconsistencies.

EXPERIMENTAL PROCEDURE

Materials—Chromatographically pure PR-ATP was a gift from B. N. Ames, University of California. All other chemicals were obtained from the sources previously listed (7). Pure PR-ATP synthetase was prepared from the strain TA2165 by our new method (7) or from the histidine Enil or 01842 mutants (8) as noted. TA2165 enzyme was stored in liquid nitrogen (7) whereas Enil enzyme was stored in 50% glycerol at 50°C (3); 01842 was used immediately.

Methods—TA2165 enzyme was transferred in all cases into the incubation buffers by Sephadex G-50 gel filtration at room temperature while E11 was done by an 8-fold dilution from storage. The standard buffer is composed of 0.10 m NaCl, 0.01 m Tris, 0.5 mm EDTA, and 1 mm DTT adjusted to either pH 7.5 with Heps or pH 8.5 with HCl. Unless otherwise noted, all pH measurements were taken at room temperature. Enzyme was incubated before assay for at least 90 min when at 0°C and for 30 min when at 37°C in those cases where equilibrium was desired.

Standard Assay—The standard assay is based on that of Voll...
et al. (9) and is as follows. A 3-ml aliquot of 0.30 M KC1, 0.20 M Tris, and 0.020 M MgCl2, adjusted to pH 8.50 with HCl, was diluted to 5.34 ml with water. To this were added 300 ml of 0.1 M ATP which had been titrated to pH 8.5 with Tris and 11 units of yeast inorganic pyrophosphatase. The pH was then readjusted, if necessary, to 8.50. A 15-ml aliquot of 0.01 M PRPP(Na4) was added to 300 ml of the above solution just before each assay. For most enzyme inhibition studies, a small aliquot of concentrated histidine which had been adjusted to pH 8.5 was also added. Enzyme was added to start the reaction at 25°C which was monitored by the absorbance increase at 290 nm due to the formation of PR-ATP (10). Volumes of enzyme smaller than 1 ml were pipetted with a 5-ml microsyringe (Hamilton Co., Whittier, California). Our unit of activity is defined as a change in absorbance of 0.10 per 5 min and corresponds to 1.75 moles of product per min based on an extinction coefficient of 3.6 X 105 for PR-ATP (11). Assays were obtained on a Gilford 2000 utilizing a full scale sensitivity of 0.2 absorbance units.

Product inhibition was studied in the standard assay by prior addition of small aliquots of concentrated PR-ATP. The PR-ATP molarity first was determined by the reverse pyrophosphorolytic reaction in the absence of inorganic pyrophosphatase on the basis of the PR-ATP extinction coefficient.

Alternate Assays—In one alternate assay, enzyme was incubated for 10 min in the presence of ATP in the assay solution by adding the PRPP last in the above procedure. In another alternate assay, enzyme was incubated in the presence of PRPP for 1 min by mixing all of the above components in the stated quantities and order except that ATP was excluded. Reaction was initiated by the addition of 15 ml of 0.1 M ATP.

Binding Studies—Equilibrium dialysis (12) was carried out overnight at 4°C in standard buffer containing 50 mM Na2HPO4, titrated to pH 7.5 with HCl. 0.144 ml enzyme (5.2 mg per ml) was prepared by prior dialysis at 4°C for one day. Treated No. 20 dialysis tubing (Union Carbide), 0.3-ml cells, and [G-3H]histidine (1 mmole = 48,000 cpm; New England Nuclear) were used. Aliquots measured by gravimetric technique were counted by liquid scintillation spectrometry (Packard Tri-Carb).

Enzyme fluorescence titration studies of histidine binding were carried out according to the method of Blasi et al. (8). About 100 pg of enzyme in 3 ml of 0.35 M KC1 and 0.01 M Tris adjusted to pH 7.5 with HCl were titrated with successive small aliquots of concentrated histidine which had been adjusted to pH 7.5. At each point the relative fluorescence at 345 nm (9-nm slit) was determined with 280-nm excitation (4-nm slit) on a Hitachi Perkin-Elmer MPF-2A fluorimeter with thermostated cell assembly at 20°C. The base-line fluorescence was established in a similar titration without enzyme.

The titration also was conducted in the presence of 0.5 mM PRPP(Na4), 10 mM MgCl2, and 1.5 units of yeast inorganic pyrophosphatase after prior incubation with the enzyme for 1 min. A titration in the presence of 10 mM ATP and 20 mM MgCl2 was possible by using 285-nm excitation and a somewhat larger concentration of enzyme. A similar titration was performed in the last case at pH 8.5 also.

RESULTS

Standard Assay after Some Different Preincubation Conditions—Several different shapes of product versus time curves were obtained when identical concentrations of PR-ATP synthetase which had been incubated under different conditions were assayed. Fig. 1 shows some typical assay curves. Linear assays were obtained for enzyme in the prior presence of histidine or at 37°C, but not at 0°C in the prior absence of histidine. The lag phase demonstrated that the enzyme gained activity for several minutes following addition to the assay buffer after which a linear product versus time curve results. The linear limiting activity of the 0°C zero histidine enzyme, which was lower than that resulting from the other preincubation conditions, was measured in the activity plots to be discussed below.

Reversible Temperature-dependent Activation—The rate of the transformation of the 0°C form to a higher temperature form of the enzyme is shown in Fig. 2. At time zero, the temperature of a solution of the enzyme in a small test tube was raised from 0°C to 22°C. A slow activation of PR-ATP synthetase occurred. When after 75 min no further increase in activity was observed, the same enzyme solution was transferred back to an ice bath. Aliquots were taken as before for assay at 25°C. A slow loss of activity occurred until the activity reached its initial (time zero) level. PR-ATP synthetase undergoes a slow, apparently reversible, temperature-dependent activation.

The kinetics of activation from the "less active" 0°C form of the enzyme to a "more active" 22°C form of the enzyme was first order as was the kinetics of the reverse process (semilog graphs not shown). The half-life for the 0 → 22°C transition was 8.9 min, while that for the 22 → 0°C transition was 17.6 min.

Effect of Temperature on Extent and Rate of Activation—The effect of temperature on the activation process is reported in Fig. 3. Little activation occurred between 0 and 11°C, substantial activation occurred between 14 and 37°C. Enzyme denaturation was apparent at 42°C and made the determination of the final activity level difficult. Clearly, the extent of activation...
The rate of the activation process was also dependent upon the temperature. The rates obtained from semilog plots were plotted in an Arrhenius plot (not shown). The activation energy for the temperature-dependent activation was 14.7 ± 1.3 Cal per mole. At 25°C, a ΔF° of 19.6 Cal per mole, a ΔH° of 14.1 ± 1.3 Cal per mole, and a ΔS° of -18.5 ± 4.4 cal per degree mole were calculated.

Effect of pH on Temperature-dependent Activation—The effect of pH on the temperature activation of PR-ATP synthetase was investigated. There was essentially no pH effect on the activation process over the pH range 7.5 to 8.4 measured at 25°C. This eliminated a pH change as the source of the activation process (the pH for this Tris buffer system decreased nearly 0.5 pH units when the temperature was raised from 0 to 25°C). The slow activation also occurred in N,N'-bis-2-hydroxyethyl glycine buffer at pH 8.5 in the same manner as in the Tris buffer.

Conformational Probes—The optical rotation of a solution of PR-ATP synthetase was measured at 233 nm as the solution warmed from 0 to 25°C. No changes were detected. No detectable differences in ultraviolet spectra or fluorescence emission spectra were observed between the “activated” and “nonactivated” forms of PR-ATP synthetase. Probably no major changes in subunit structure occur upon activation, but rather minor shifts of important residues which affect gross spectroscopic parameters only slightly.

Activation of PR-ATP Synthetase at 0°C by Substrates—Zero-degree enzyme was found to be activated by substrate ligands also. The effect of ATP is shown in Fig. 4. The addition of 10 mM ATP to the enzyme at 0°C resulted in a 2-fold activation over a 90-min time period. The kinetics of the ATP-dependent activation appeared first order and had a half-life of 19 min.

The activation of PR-ATP synthetase at 0°C by ATP was concentration dependent (Table I). The ATP concentration for half-maximal activation at 0°C was about 500 μM which is similar to the K₅₀ of the enzyme for ATP (2, 3). When PR-ATP synthetase was incubated with other substrate ligands at 0°C, an activation also was observed (Table I).

Activation of PR-ATP Synthetase at 0°C by Histidine—The histidine-dependent activation of 0°C enzyme seen in Fig. 1 was investigated under equilibrium conditions as shown in Fig. 5. The specific activity of the fully histidine-activated enzyme was about 2.5 times that of the unactivated enzyme. The activation process was cooperative with a Hill constant of 2.4 and the midpoint occurred at 0.071 mM histidine. Assays became increasingly more linear at zero time after addition of enzyme as the histidine incubation concentration was increased.

Dependence of Activation on Protein Concentration—The histidine activation factor for the 0°C enzyme was variable in different experiments, depending upon the enzyme concentration. The possibility of an enzyme dissociation could be checked by carrying out a dilution study in the presence and absence of incubation histidine by assayig larger aliquots of the more dilute enzyme. There was no effect on the apparent specific activities of 0°C enzyme arising from varying the incubation concentration over the range 6 to 0.00 mg per ml of enzyme. The histidine activation factor was about 1.8 in this experiment. The variable histidine activation factor was not related to the enzyme concentration during preincubation.

Another possible concentration effect might arise from the amount of enzyme actually in the assay solution. Fig. 6 shows an experiment in which increasing aliquots of a single enzyme solution incubated at 0°C in zero histidine standard buffer were...
assayed. The specific activity decreased as the enzyme concentration in the assay solution increased. A similar experiment on histidine-preactivated enzyme showed no such assay dilution effect. The decrease in activity of the zero histidine enzyme might be due to a transformation of the enzyme from an active dissociated state at low assay concentrations to an inactive aggregated state at high assay concentrations. This possibility was checked by analyzing the shape of the supposed dissociation curve as discussed in the legend to Fig. 6. These calculations show that all reasonable permutations for a dissociation-association explanation are discarded. Furthermore, $K_a$ for 0° zero histidine enzyme in the assay solution previously was determined (5) by the Cohen method at a layered initial concentration amount of ATP was found to decrease almost to 1.0 at 5 mM ATP. Thus, an increase in either temperature or ATP concentration seems to induce a similar activity state of the enzyme.

**Feedback Inhibition of Enzyme Activated by Histidine or High Temperature**—To investigate further the effect of temperature upon PR-ATP synthetase, initial rates were measured for 0 and 23° forms of the enzyme at different assay temperatures. An Arrhenius plot is shown in Fig. 8. The two curves are nearly parallel and both show a sharp break between 16 and 20°. Calculation of the energy of activation for product formation from the 4 to 16° arm of the curve for the 0° enzyme form is 43.0 Cal per mole, respectively. The energy of activation calculated in the 20 to 40° arm of the curve for the 0° enzyme form is 18.3 Cal per mole per 280 nm absorbance unit of enzyme.

**Interdependence of Substrate and Temperature-dependent Activation Processes**—The extent of temperature activation was diminished by previous ATP activation at 0° (cf. Fig. 8). The ratio of the activation at 23° to that at 0° in the presence of increasing amounts of ATP was found to decrease almost to 1.0 at 5 mM ATP. Thus, an increase in either temperature or ATP concentration seems to induce a similar activity state of the enzyme.

The most straightforward explanation for the apparent dilution activation is that it is caused by product inhibition. The onset of product inhibition under these conditions can be demonstrated by pre-adding PR-ATP to the standard assay. Fig. 7 presents the inhibition observed using 0° zero histidine enzyme. Significant product inhibition occurs when about 0.1 absorbance unit of PR-ATP has been formed. It is to be remembered that 0° zero histidine enzyme does not attain full activity for several minutes. Thus, product inhibition sets in before all of the enzyme has been activated when a high concentration of enzyme is assayed. The opposing activation-inhibition effects produce approximate linearity in the assay for a period of time. The enzyme appears to have a lower specific activity than an identical sample at lower concentration, but this is an artifact. The maximal specific activity which 0° zero histidine enzyme can exhibit under these assay conditions consequently is uncertain. The highest observed has been about 1700 units per ml per 280 nm absorbance unit of enzyme.

**Arrhenius Plot for Temperature-dependent Forms of PR-ATP Synthetase**—To investigate further the effect of temperature upon PR-ATP synthetase, initial rates were measured for 0 and 23° forms of the enzyme at different assay temperatures. An Arrhenius plot is shown in Fig. 9. The two curves are nearly parallel and both show a sharp break between 16 and 20°. Calculation of the energy of activation for product formation from the 4 to 16° arm of the curve for the 0° enzyme form is 43.0 and 42.2 Cal per mole, respectively. The energy of activation calculated in the 20 to 40° arm of the curve for the 0° enzyme form is 18.3 Cal per mole per 280 nm absorbance unit of enzyme.

The similarity of the slopes and the breaks in the Arrhenius plots indicated by the arrow (5). Enzyme behavior as a hexamer of 8.7 $s_{20,w}$.
FIG. 7. Product inhibition of the 0° zero-histidine enzyme. 
TA2165 enzyme preincubated in standard buffer at pH 7.5 was 
assayed in the presence of increasing amounts of PR-ATP pre-
added in the standard assay procedure. The reciprocal of initial 
velocity is plotted versus concentration of PR-ATP. One-half 
inhibition occurred at 0.17 mM PR-ATP. This corresponds to 
an absorbance at 290 nm of 0.6.

specific activity from 0° histidine-activated enzyme (cf. Fig. 1). 
However, a difference between the two 37° enzymes became 
apparent when histidine feedback inhibition was studied. Fig. 
10 shows a plot of the residual activity of enzyme preincubated 
in the presence and absence of histidine as a function of the 
amount of histidine in the assay buffer. The histidine-preincu-
bated enzyme was inhibited 100% by 0.2 mM assay histidine 
while the histidine naive enzyme retained 12% activity even 
in the presence of 8.7 mM assay histidine. This residual activity 
decreased to zero after several minutes in the high histidine assay 
solution, thus giving a concave down assay trace as shown by the 
dashed line in Fig. 1. In other words, 37° zero histidine-incu-
bated enzyme cannot be totally inhibited instantaneously, but 
does relax within a few minutes under assay conditions to a form 
which is totally inhibited.

Three Enzyme States Dependent on Temperature and Histidine—
Table II summarizes some kinetic behavior in the standard assay 
of PR-ATP synthetase incubated in standard buffer at pH 7.5 
under the four conditions of Fig. 1. The histidine-preincubated 
enzyme is more cooperatively inhibited by histidine in the assay 
both at 0 and 37°. The apparent specific activity for the 0° 
zero histidine enzyme is variable but clearly lower than for the 
other enzyme states. On the basis of Table II there are three 
discernible states of the enzyme, one each at 0 and 37° in the 
absence of histidine and the one resulting from histidine incu-
bation at both temperatures. Very similar results were ob-
tained from enzyme incubated at pH 8.5 under otherwise similar 
conditions.

Assay Order of Addition and Feedback Inhibition—The results 
above were obtained with the “standard assay,” which involved 
addition of enzyme to initiate the reaction. However, the time 
lag phenomenon and substrate activation indicated that the 
kinetic behavior of PR-ATP synthetase would also depend on
the order of addition of substrate and enzyme to the assay solution. This was found to be the case as shown in Fig. 11. The histidine feedback inhibition patterns for similar solutions of enzyme were obtained in one case by initiating the reaction with PRPP (ATP incubation) and in the other case by initiating it with ATP (PRPP incubation). Varying levels of histidine were in the assay buffer and enzyme was added next to last. Enzyme preincubated in the presence of ATP could not be totally inhibited by high levels of histidine and the inhibition response was noncooperative. On the other hand, PRPP-preincubated enzyme was essentially totally inhibitable in a highly cooperative manner. Thus there are at least two substrate-dependent enzyme states possible.

**Histidine Equilibrium Binding Studies** The results of Fig. 5 were the kinetic consequence of histidine binding, as were all of the above feedback inhibition data. It was desirable to observe directly histidine binding under equilibrium conditions. Fig. 12 shows an equilibrium dialysis curve of histidine binding to the enzyme at low temperature. It can be seen that the binding is very cooperative with a Hill constant of 3.1 and $K_i$ of 0.040 mM.

![Fig. 10. Feedback inhibition of 37°C enzyme states. TA2165 enzyme was incubated at 37°C and pH 7.5 in the standard buffer, in the presence or absence of 0.4 mM l-histidine. One micro-liter was quickly assayed by the standard method in solutions containing increasing concentrations of histidine. Time zero activity is plotted as a function of assay histidine. Assays of the zero-histidine-incubated enzyme were concave down at high histidine concentrations as indicated by the dashed line in Fig. 1.](image)

**Table II**

| Incubation conditions | 0° | 37° |
|-----------------------|----|-----|
| No histidine | 0.4 mM histidine | No histidine | 0.4 mM histidine |
| $n_H$ (mm) | 1.9 | 2.8 | 1.1 | 3.0 |
| $K_i$ (mm) | 0.040 | 0.037 | 0.052 | 0.042 |
| Specific activity (units/ ml/A) | (1700) | 3200 | 3200 | 3200 |
| Assay shape | Lag | Linear | Inverse lag | Linear |
| Feedback Inhibition | 100% | 100% | 88% | 100% |

![Fig. 11. Effect of order of addition of substrates on feedback inhibition. The 0° zero-histidine form of the TA2165 enzyme was preincubated at 25°C in the assay solution containing the indicated substrate but not the other substrate, as described in detail under "Experimental Procedure." The assay was initiated by the addition of the second substrate. The slope at time zero was taken for measurement. The midpoint of inhibition $K_i$ and cooperativity constant $n$ were determined on separate Hill plots in both cases. Enzyme prepared by an older method was used (8). The small residual apparent activity for the PRPP preincubated enzyme is probably an artifact caused by contaminating histidase, which was discovered later (7).](image)

![Fig. 12. Equilibrium dialysis curve of histidine binding to PR ATP synthetase. O2165 enzyme (5.2 mg per ml), prepared by an old method (8), was equilibrated in standard buffer at pH 7.5 containing 50 mM phosphate at 4°C with tritium-labeled histidine, as detailed under "Experimental Procedure." Binding saturates at 5.2 g moles of histidine per 216,000 grams of enzyme. The inset gives the Hill plot. The binding is not comparable to that in Fig. 13 for reasons discussed in the text.](image)
shown. The bottom two curves were obtained in the presence of histidine in standard buffer at pH 7.5 and 25° is a temperature-dependent process is reversible and can be influenced by substrates and the allosteric effector histidine. Activation of 0° enzyme occurs primarily above 11° under incubation conditions and appears to be essentially complete at 37°. The physical basis for the difference between 0° and 22–37° enzyme was elucidated in an accompanying paper (5). Low temperature enzyme (4–8°) exists in a continuous distribution of aggregated species dependent upon protein concentration while high temperature enzyme (22–37°) exists predominately as a hexamer.

Different 0° forms of the enzyme which depend on the presence or absence of histidine were detected by kinetic analysis. The physical basis for these forms also has been elucidated elsewhere by the demonstration that 0° histidine-activated enzyme exists as the hexamer (5). Since it exhibits a pronounced lag upon assay, the 0° indefinitely aggregated enzyme is initially inactive or of greatly reduced activity. In addition, this enzyme never achieves an activity state during assay which exhibits kinetic parameters similar to those of the histidine-activated enzyme. Nevertheless, sedimentation velocity analysis of the two forms of the active enzyme-substrate complex (5) demonstrated that both forms of the enzyme are hexamers under assay conditions. Apparently, indefinitely aggregated low activity 0° zero histidine enzyme reassociates within several minutes in the assay buffer to give an active hexamer, this process being the cause of the kinetic lag. An apparent dilution activation of 0° zero histidine-inactivated enzyme was shown to be an artifact caused by product inhibition and the time lag. Since both the cooperativity of histidine inhibition and the specific activity are significantly decreased, it is as if the rapidly assembled histidine-naive hexamer does not contain properly “interlocked” subunits.

Fig. 13. Fluorescence enhancement titration of histidine binding. The increase in fluorescence of the TA165 enzyme in the presence of histidine in standard buffer at pH 7.5 and 25° is shown. The bottom two curves were obtained in the presence of histidine at pH 7.5 and 25° is a temperature-dependent process is reversible and can be influenced by substrates and the allosteric effector histidine. Activation of 0° enzyme occurs primarily above 11° under incubation conditions and appears to be essentially complete at 37°. The physical basis for the difference between 0° and 22–37° enzyme was elucidated in an accompanying paper (5). Low temperature enzyme (4–8°) exists in a continuous distribution of aggregated species dependent upon protein concentration while high temperature enzyme (22–37°) exists predominately as a hexamer.

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Dilution activation of Escherichia coli PR-ATP synthetase has been reported by Kryvi and Klungsoyr (14). The E. coli enzyme may be different in that no assay lag has been reported, and the dilution activation occurred after incubation in an assay solution containing ATP, which stabilizes the hexamer form of the Salmonella enzyme (5). Therefore, it cannot with certainty be concluded that the dilution activation phenomena of the two enzymes are the same.

Two different 37° forms of the enzyme which depend upon histidine were detected by kinetic analysis. Both of the 37° enzymes were shown in a previous paper (5) to be hexamers. Thus, the kinetic differences between the two 37° enzymes apparently lie in different slowly interconvertible conformational states of the hexamer which regulate both the cooperativity of histidine inhibition and residual activity at high histidine levels. This residual activity is not due to a partial desensitization toward histidine as a result of “aging” as has been observed before (1), since the enzyme does become inhibited after a few minutes. There appears to be no difference on the basis of the kinetic studies presented here or the previously mentioned physical studies between histidine-ligated enzyme at 0° or 37°. Histidine appears to stabilize one particular hexameric state of the enzyme against temperature changes.

In addition to the enzyme states which depend upon incubation temperature and histidine conditions, there are states which depend upon prior substrate action. Activation of 0° enzyme occurs in the presence of ATP, PRPP, and PPI. This probably is the result of stabilization of hexameric enzyme, since both ATP and PRPP have been shown to produce hexamers from dimeric enzyme under other conditions (5). Incubation of the enzyme at 25° and pH 8.5 in the presence of ATP abolishes cooperativity of histidine inhibition and induces a residual enzymatic activity at high histidine levels. This behavior is very similar to that resulting from incubation in zero histidine at 37°, and may indicate that the two sets of conditions promote the same hexameric enzyme state.

Enzyme incubated at 25° is inhibited totally in a very cooperative manner by histidine. This behavior is similar to that resulting from histidine incubation. Thus, there is compelling evidence only for three different slowly interconverting states on the basis of the kinetic evidence presented here.

Equilibrium Binding of Histidine—Slightly less than 6 g moles of histidine are bound at 4° at saturation by 216,000 g of the
enzyme, in approximate agreement with the expected hexameric structure. This is in contrast to only 3 moles of histidine bound to the E. coli enzyme (15). The binding was a highly cooperative process, but histidine binding at 4°C is a very complex process since the unliganded enzyme exists in an indefinitely aggregated state (5). The apparent Hill coefficient and binding constant will depend upon the enzyme concentration (16, 17). Thus, binding curves obtained under such conditions cannot be compared simply to those obtained at high temperature or in the presence of other ligands.

The result of the histidine binding study as determined by fluorescence titration at 25°C was similar to that obtained by Blasi et al. (8). ATP reduced the cooperativity of the histidine effect and increased the apparent histidine dissociation constant in both kinetic and equilibrium measurements. PRPP had precisely the opposite effects. Since neither the conditions of incubation, the enzyme preparations, nor the measured processes were identical, the lack of precise agreement in Hill coefficients and dissociation constants for the histidine effects on kinetic and equilibrium phenomena is not surprising. The qualitative results, however, are in agreement.

Possible Metabolic Role of Time-dependent States—Slow interconversions of kinetic states of PR-ATP synthetase have been noted briefly by others. Klungsoyr and Atkinson (15) observed a lag in inhibition depending upon the extent of PR-ATP accumulation before the addition of histidine to the E. coli enzyme. Bell and Koshland (3) observed a lag in inhibition by histidine for the S. typhimurium enzyme. The above complexities in the enzyme behavior explain many of the apparent literature contradictions mentioned in the introduction. Further study may reveal that the different types of substrate kinetics arise from the different enzyme states. The histidase contaminant would be particularly confusing since the effects would be irreproducible because of its complex pattern of both activation and deactivation by DTT and EDTA (18). Previous residual “PR-ATP synthetase” activity at high levels of histidine was sometimes authentic and sometimes due to the histidase contaminant.

Possible Metabolic Role of Time-dependent States—Slow time-dependent interconversions of conformational states have been observed in a number of enzymes (19), a process which Frieden (20) has referred to as “hysteresis” and Alpers and Paulus (21) as “preconditioning.” Yet the need for such a time-dependent change is not immediately apparent, nor is it clear that it has any in vivo importance. PR-ATP synthetase may provide a clue in this regard.

One of the substrates of PR-ATP synthetase, PRPP, is used in the biosynthesis of histidine, tryptophan, nicotinamide nucleotides, and purine and pyrimidine nucleotides and deoxyribonucleotides (22). White et al. (23) has discussed the disposition of PRPP in vivo, and points out that about 98% is normally utilized in nucleotide and deoxyribonucleotide synthesis. Perturbations in the utilization rate of PRPP by either amino acid pathway will therefore normally have little effect on the PRPP pool. In contrast, a lower utilization rate of PRPP for nucleotide and deoxyribonucleotide biosynthesis could greatly raise the PRPP concentration (albeit the increase will be modulated by phosphoribosylpyrophosphate synthetase effectors (24)). An increase in PRPP concentration would increase the rate of histidine biosynthesis. From the properties described in these papers, high histidine and PRPP levels would convert the enzyme into the cooperatively totally inhibitable state. This would eventually completely shut off PR-ATP synthetase. Thus histidine biosynthesis could be coupled to RNA and DNA production via the PRPP pool.

Under growth conditions limited by the available histidine, a high level of ATP and a low level of PRPP would exist during unrestricted RNA and DNA synthesis. This would favor the noncooperatively incompletely inhibitable form of PR-ATP synthetase. This state of the enzyme is similar to that described by Klungsoyr et al. (25), at an “energy charge” of 1.0. A rapid increase in the histidine pool would affect this state of the enzyme by decreasing catalysis, but only noncooperatively and only to the extent of 88% (or less). It should be noted that because PR-ATP synthetase activity is not rate-limiting in histidine biosynthesis (26) this residual activity may be enough to maintain more than 12% mass flow through the pathway. Should the rapid histidine increase turn out to be just a pulse of histidine which was soon dissipated, the enzyme would not have completely shut down catalysis and there would be some biosynthetic intermediates in the pathway along the way to histidine. Such a delay in cutting off biosynthesis may be useful in preventing erratic fluctuations, particularly in very long pathways (nine enzymatic steps follow PR-ATP synthetase) where the time of synthesis may be great. However, if the high histidine environment persisted, the enzyme would adapt to the completely inhibitable enzyme. The concomitant shift to highly cooperative histidine inhibition would allow immediate higher order catalytic response should the histidine level seriously drop again.

Thus the time-dependent characteristics of this enzyme and similar enzymes might be a device for shifting between more sensitive and less sensitive enzyme forms to avoid overshoots due to erratic fluctuations of a key metabolite. In addition a time-dependent conformational change has been suggested as an important regulatory device in sensory systems such as chemotaxis of microorganisms (27). Time-dependent changes do provide a further regulatory device and it seems unlikely that their widespread occurrence is without physiological significance.

REFERENCES
1. Martin, R. G. (1963) J. Biol. Chem. 238, 257
2. Whitfield, H. J., Jr. (1971) J. Biol. Chem. 246, 899
3. Bell, R. M. and Koshland, D. E., Jr. (1971) Bioorg. Chem. 1, 409
4. Bell, R. M., and Koshland, D. E., Jr. (1970) Biochem. Biophys. Res. Commun. 38, 539
5. Parsons, S. M., and Koshland, D. E., Jr. (1974) J. Biol. Chem. 249, 4110-4126
6. Rossotti, F. J. C., and Rossotti, H. (1961) The Determination of Stability Constants, p. 328, McGraw-Hill, New York
7. Parsons, S. M., and Koshland, D. E., Jr. (1974) J. Biol. Chem. 249, 4104-4109
8. Blasi, F., Aloi, S. M., and Goldberg, R. F. (1971) Biochemistry 10, 1409
9. Voll, M. J., Appella, E., and Martin, R. G. (1967) J. Biol. Chem. 242, 1769
10. Ames, B. N., Martin, R. G., and Garry, B. J. (1961) J. Biol. Chem. 236, 2019
11. Smith, D. W. E., and Ames, B. N. (1965) J. Biol. Chem. 240, 3056
12. Myer, Y. P., and Sheehman, J. A. (1962) Biochim. Biophys. Acta 56, 361
13. McPhee, P. (1971) Methods Enzymol. 13, 23
14. Kerpyn, H., and Klungsoyr, L. (1971) Biochim. Biophys. Acta 235, 429
15. Klungsoyr, L., and Atkinson, D. E. (1970) Biochemistry 9, 2021
16. Wyman, J., Jr. (1964) Advan. Protein Chem. 19, 224

2 S. M. Parsons and D. E. Koshland, Jr., unpublished observation.
17. Weber, G. (1972) *Biochemistry* **11**, 864
18. Klee, C. B. (1972) *J. Biol. Chem.* **247**, 1308
19. Frieden, C. (1971) *Annu. Rev. Biochem.* **40**, 653
20. Frieden, C. (1970) *J. Biol. Chem.* **245**, 5788
21. Alpers, J. B., and Paulus, H. (1971) *Nature* **233**, 478
22. Sorensen, C. (1970) *Handbook of Biochemistry*, Second Ed., The Chemical Rubber Co., Cleveland
23. White, M. N., Olstowy, J., and Switzer, R. L. (1971) *J. Bacteriol.* **108**, 122
24. Switzer, R. L., and Sogin, D. C. (1973) *J. Biol. Chem.* **248**, 1063
25. Klungsoyr, L., Hagemen, J. H., Fall, L., and Atkinson, D. E. (1968) *Biochemistry* **7**, 4035
26. Brenner, M., and Ames, B. N. (1971) in *Metabolic Pathways V* (Greenberg, D. M., and Vogel, H. J., eds.) Chap. II, Academic Press, New York
27. Macnab, R. M., and Koshland, D. E., Jr. (1972) *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2509
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J. Biol. Chem. 1974, 249:4110-4118.

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