Interaction between Energy Charge and Product Feedback in the Regulation of Biosynthetic Enzymes. Aspartokinase, Phosphoribosyladenosine Triphosphate Synthetase, and Phosphoribosyl Pyrophosphate Synthetase*

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ABSTRACT: The prediction that energy charge (half the average number of anhydride-bound phosphate groups per adenosine moiety) and end-product concentration should jointly control biosynthetic metabolism has been tested with three enzymes from *Escherichia coli* that catalyze first steps in biosynthetic sequences: lysine-sensitive aspartokinase (EC 2.7.2.4), phosphoribosyladenosine triphosphate synthetase, and phosphoribosyl pyrophosphate synthetase (EC 2.7.6.1). Slopes of the curves showing the rates of the reactions catalyzed by aspartokinase and phosphoribosyl pyrophosphate synthetase as functions of energy charge increase with charge; rates are low at charge values below about 0.7, and the curves are concave upward and steeply rising in the charge interval 0.8–1.0. The addition of end products (lysine for aspartokinase; a mixture of nucleotides and tryptophan for phosphoribosyl pyrophosphate synthetase) depresses the reaction rates and decreases the upward concavity of the curves. The corresponding curve for phosphoribosyladenosine triphosphate synthetase in the absence of end product (histidine) is not concave upward, but histidine interacts synergistically to produce curves like those seen with the other enzymes. This behavior seems adapted to regulate biosynthetic processes in response to both metabolic demand for end products and cell energy resources

Product feedback modulation of enzymic behavior, first demonstrated by Umbarger (1956) and Yates and Pardee (1956), has been found to be a general means of regulation of biosynthetic sequences (for reviews, see Moyed and Umbarger (1962), Stadtman (1966), and Atkinson (1966)). Such sequences may also be regulated by the energy charge of the adenylate system (Atkinson and Fall, 1967). The preceding paper (Atkinson, 1968) suggests that both of these types of control may frequently affect the same enzyme.

This paper describes simultaneous response to energy charge and feedback modifier observed in studies on lysine-sensitive aspartokinase (ATP + L-aspartate → ADP + 4-phospho-L-aspartate, EC 2.7.2.4), PR-ATP[1] synthetase (ATP + PRPP → PR-ATP + pyrophosphate), and PRPP synthetase (ATP + D-ribose 5-phosphate → AMP + PRPP, EC 2.7.6.1). All three enzymes were obtained from *Escherichia coli*.

Materials and Methods

Aspartokinase (Lysine Sensitive). ASSAY. Aspartokinase activity was assayed spectrophotometrically by following TPNH oxidation in the presence of excess aspartate semialdehyde dehydrogenase. The standard assay mixture (1 ml) contained: 80 mM Na-Bicine (pH 8.5), 20 mM 2-mercaptoethanol, 0.2 mg of aspartate semialdehyde dehydrogenase preparation, aspartokinase, 20 mM L-aspartate, 10 mM $MgCl_2$, and 2 mM ATP. The concentrations of these last three compounds were varied in kinetic experiments. NaOH was used for pH adjustments. Aspartokinase and aspartyl semialdehyde dehydrogenase were passed through a Sephadex G-25 column in 20 mM sodium phosphate (pH 7.2) and 30 mM 2-mercaptoethanol to remove NH_4^+ and K^+ ions. The reaction was initiated by addition of aspartokinase or ATP.

The aspartate semialdehyde dehydrogenase (EC 1.2.1.11) used in the aspartokinase assay was a slightly purified preparation from *E. coli*, Crookes strain, grown on glucose–salts minimal medium. The cells were broken, centrifuged, treated with protamine, heated, and centrifuged as described for the preparation of aspartokinase. The protein fraction soluble at 50% saturation with $(NH_4)_2SO_4$ and precipitating at 70% saturation was passed through a Sephadex G-200 column in a solution containing 30 mM 2-mercaptoethanol and 20 mM potassium phosphate (pH 7.2). Fractions containing the highest activity were pooled and concentrated by precipitation with $(NH_4)_2SO_4$ at 70% saturation. The precipitate was dissolved in 50 mM

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: PR-ATP, *N*-1-(5-phosphoribosyl)adenosine 5′-triphosphate; PRPP, 5-phospho-D-ribosyl α-pyrophosphate; Bicine, *N,N*-bis(2-hydroxyethyl)glycine.

potassium phosphate (pH 7.2) containing 30 mM 2-mercaptoethanol. The specific activity of the preparation was 7 μmoles/min per mg of protein when assayed in the reverse direction (oxidation of aspartate semialdehyde to 4-phosphoaspartate). This preparation contained no homoserine dehydrogenase activity. The activity of this enzyme was not affected by NH_4^+ or K^+ ions under the conditions used for study of the effects of these ions on aspartokinase.

PREPARATION OF ASPARTOKINASE. *E. coli*, Crookes strain, was grown in a 12-l. New Brunswick fermenter at 37.5° with maximal aeration and agitation in a glucose–mineral medium supplemented with 20 mM DL-methionine. Growth was followed turbidimetrically, and cells were harvested 1 hr after the end of exponential growth. The cells were washed in 50 mM potassium phosphate buffer (pH 7.2) containing 30 mM 2-mercaptoethanol and stored at −20°.

Thawed cell paste (45 g) was suspended in 135 ml of 50 mM potassium phosphate (pH 7.2) containing 30 mM 2-mercaptoethanol, and the cells were broken with a Bronson Sonifier. The temperature was maintained below 5° by use of an ice–salt bath. The extract was centrifuged at a maximum acceleration of 105,000*g* for 25 min. The supernatant solution was decanted and treated with an equal volume of a freshly made 1% solution of protamine sulfate adjusted to pH 5.4 with KOH. This mixture was stirred for 16 min at 0° and centrifuged for 15 min at 14,600*g*. The supernatant solution was made 10 mM in L-lysine by addition of 0.01 volume of a 1 M solution. After thorough mixing, the solution was placed in a water bath at 54° for 32 min. After cooling to 5°, the solution was centrifuged for 15 min at 14,600*g*. The resulting supernatant solution was slightly cloudy and had a pH of 6.8.

Solid ammonium sulfate was added to 35% saturation. The solution was stirred for 16 min and centrifuged as before. The precipitate was dissolved in a small volume of the standard phosphate–mercaptoethanol buffer. Ammonium sulfate was added to bring the supernatant solution to 50% saturation. The resulting precipitate was dissolved in a small volume of standard mercaptoethanol buffer and used in the experiments described. The crude extract had a specific activity of 0.097 μmole/min per mg of protein, and was inhibited 25–35% by 10 mM lysine. The 50% ammonium sulfate precipitate had a specific activity of only 0.15, but was inhibited 100% by 10 mM lysine. Thus other aspartokinases (Stadtman *et al.*, 1961; Patte *et al.*, 1967) had been removed. The preparation was free of homoserine dehydrogenase.

PR-ATP Synthetase. ASSAY. The assay procedure was that of Voll *et al.* (1967), except that lower levels of PRPP were used (125 and 250 μM). Initial rates were estimated with a recording spectrophotometer (Gilford Model 2000 or Beckman DB with Sargent recorder). Pyrophosphatase was prepared from yeast (Heppel, 1955).

ISOLATION OF MUTANT STRAINS. Histidine-requiring mutant strains of *E. coli* were obtained from wild-type strain C-600-1 by treatment with methylnitronitrosoguanidine, followed by standard penicillin-enrichment procedures. The enzymes of the histidine biosynthetic sequence are derepressed when strains blocked at some point in the pathway are grown on glucose–mineral medium supplemented with histidinol (Smith and Ames, 1964). Under these conditions, cells of our histidine-requiring strain X-9 contained PR-ATP synthetase, the first enzyme of the sequence, at 10–20 times the usual level in the parental strain.

Mutant strains with decreased sensitivity to histidine feedback regulation were isolated by selecting for colonies which grew rapidly on agar medium containing thiazolealanine (Moyed, 1961) after treatment with methylnitronitrosoguanidine. Selected mutant strains were then again treated with the mutagen and carried through standard penicillin-enrichment procedures, selecting for colonies requiring histidine for growth. Cells of regulation-negative, histidine-requiring strain R4-1, when grown on minimal medium supplemented with histidinol, produced an enzyme that was largely insensitive to histidine. The level of the enzyme was about 20 times as high as in the regulatory mutant R4 from which the double mutant strain was derived.

PREPARATION OF PR-ATP SYNTHETASE. Cells were grown at 37° with vigorous aeration in a 20-l. fermenter, harvested by centrifugation, and washed in 10 mM imidazole–HCl buffer (pH 7.0) containing 5 mM 2-mercaptoethanol. The cells were suspended in 1.2 ml of buffer/g of wet cell paste and broken by treatment with a Bronson Sonifier. After centrifugation for 30 min at 27,000*g*, the supernatant solution was applied to a 50 mm × 100 cm Sephadex G-200 column equilibrated with the same imidazole–mercaptoethanol buffer, which was also used for elution. Fractions containing the highest activity were pooled, and 22 g of $(NH_4)_2SO_4$ was added/100 ml. The precipitate, which contained the bulk of the activity, was dissolved in the same buffer. This procedure results in about sevenfold purification from the extracts of the derepressed cells. Preparations from strain X-9 used for experiments reported in this paper had a specific activity of about 0.1 μmole/min per mg of protein, and the specific activity of preparations from double mutant strain R4-1 was about 0.3. During storage at 4°, about 30% of the activity was lost in 2 weeks.

PRPP Synthetase. ASSAY. The assay procedure was modified slightly from that described by Kornberg *et al.* (1955). Phosphoribosyl pyrophosphate produced by the synthetase was assayed by conversion into UMP. The synthetase preparation and any modifiers to be tested were incubated for 10 min at 35° in a 1-ml assay mixture containing 60 mM potassium phosphate buffer (pH 7.5), 5 mM $MgCl_2$, 1.0 mM ATP, and 2.0 mM ribose 5-phosphate. The concentrations of the two substrates and of $MgCl_2$ were varied as appropriate in kinetic studies. The reaction was stopped by placing the assay tube in boiling water for 45 sec. A measured portion (0.7–0.9 ml) of the cooled assay mixture was added to 100 μmoles of Tris-HCl (pH 8.5), 2.0 μmoles of $MgCl_2$, and 0.10 or 0.15 μmole of potassium orotate in a total volume of 3.0 ml and the absorbance at 295 nm was read. After the addition of 0.1 ml of yeast extract containing orotidylate synthetase (pyrophosphorylase) and

orotidylate decarboxylase, the mixture was incubated at room temperature until constant absorbance readings at 295 nm were obtained (30–45 min). The amount of PRPP synthesized was calculated on the basis of a differential molar absorbancy of 3950.

The crude preparation of orotidylate synthetase and orotidylate decarboxylase required in the assay was prepared from baker's yeast (Fleischmann). Cells were broken in a French press and the resulting preparation was centrifuged for 2 hr at a maximum acceleration of 100,000*g*. The clear central portion of the extract was removed from the tube by suction and made 70% saturated in $(NH_4)_2SO_4$. The resulting precipitate was dissolved in 0.1 M Tris-HCl (pH 8.0) and dialyzed against several changes of the same buffer. The dialyzed solution, which contained no PRPP synthetase activity, was stored at −20° in small batches until needed.

PREPARATION OF PRPP SYNTHETASE. *E. coli* B was grown aerobically in a glucose–peptone–yeast extract medium and harvested by centrifugation. The wet cell paste was resuspended in four volumes of 0.1 M potassium phosphate buffer (pH 8.0) and passed through a French press. The extract was centrifuged for 1 hr at a maximum acceleration of 100,000*g*. The supernatant solution was diluted with an equal volume of water, and the pH was adjusted to 5.4 by addition of 1.0 M potassium acetate and then to pH 5.3 by dropwise addition of 1 M acetic acid. The precipitate was obtained by centrifugation and dissolved in 100 mM potassium phosphate (pH 8.0) at about 10 mg of protein/ml. The solution was fractionated by addition of ethanol at −4°, and the fraction precipitating in the 30–50% range was dissolved in 100 mM potassium phosphate buffer (pH 8.0). After centrifugation to remove suspended insoluble material, the solution was desalted by passage through a Sephadex G-25 column. The activity is stable for at least several months when the preparation is frozen in the presence of 1% bovine serum albumin. Over-all purification from the centrifuged crude cell-free preparation was about eightfold.

Results

Aspartokinase. KINETIC CONSTANTS. Under the standard assay conditions, $(S)_{0.5}$ values (the concentration of substrate required for half-maximal velocity; thus identical with K_m when the simple first-order Michaelis treatment is applicable (Koshland *et al.*, 1966)) were: L-aspartate, 0.74 mM; ATP, 0.28 mM (rate *vs.* concentration curves were biphasic, suggesting a second kinetic response with an $(S)_{0.5}$ of about 0.75 mM); and $MgCl_2$, 0.20 mM. Under conditions of the energy charge experiments (with a charge of 1.0), assays of undialyzed enzyme preparation resulted in a normal plot of rate against ATP concentration (not biphasic), with an $(S)_{0.5}$ of 0.26 mM. Reactions were initiated by the addition of ATP or enzyme.

Paulus and Gray (1964) reported that aspartokinase from *Bacillus polymyxa* has an absolute requirement for K^+ or NH_4^+ ion, with NH_4^+ giving a higher maximal velocity. The undialyzed preparation of lysine-sensitive *E. coli* aspartokinase did not respond to addition of K^+ and only weakly to NH_4^+, probably because of the NH_4^+ carried over from $(NH_4)_2SO_4$ fractionation. After passage through a Sephadex G-25 column, the enzyme was stimulated eight- to tenfold by either NH_4^+ or K^+. At a charge value of 1.0, the level of K^+ required for half-maximal activity was about 20 mM, in good agreement with the value of 25 mM reported by Wampler and Westhead (1968). Because NH_4^+ gives half-maximal activation at a considerably lower concentration (about 2 mM), it seems likely that NH_4^+ is the physiological modifier. Corresponding values reported for the *B. polymyxa* enzyme were 30 mM for NH_4^+ and 90 mM for K^+ (Paulus and Gray, 1967).

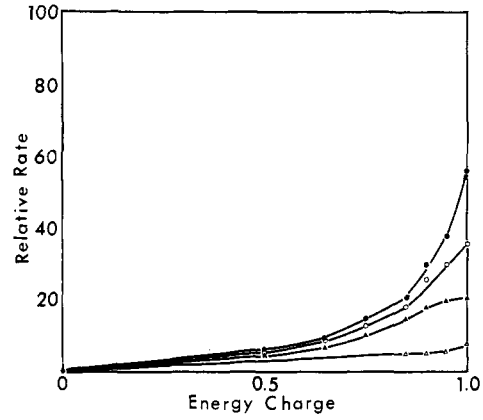

FIGURE 1: Rate of the reaction catalyzed by *E. coli* lysine-sensitive aspartokinase as a function of energy charge; interaction with lysine concentration. The size of the adenine nucleotide pool (ATP + ADP + AMP) was 4 mM. Appropriate mixtures of AMP and ATP were incubated for 30 min in 0.5 ml of 80 mM Na-Bicine (pH 8.5) containing 6 μmoles of $MgCl_2$ and 6–10 μg of adenylate kinase. Aspartokinase and aspartyl semialdehyde dehydrogenase were incubated for 15 min in 0.5 ml of the same buffer containing 10 μmoles of L-aspartate, 20 μmoles of 2-mercaptoethanol, 2.0 μmoles of NH_4Cl, and L-lysine at levels varying from 0 to 2 μmoles. The reaction was started by mixing the two solutions, and was followed spectrophotometrically at 340 nm. Rate is expressed in terms of the rate observed with a saturating level of NH_4^+ (Figure 2). Concentration of L-lysine: (●) 0, (○) 0.3, (▲) 0.5, and (△) 2.0 mM.

LYSINE INHIBITION. Freshly dialyzed enzyme was used. Concentrations in the reaction mixture were: TPNH, 0.15 mM; L-aspartate, 5 mM; ATP, 1.0 mM; and $MgCl_2$, 4.0 mM. The reaction was initiated by addition of ATP. The apparent K_I for lysine under these conditions was 0.21 mM, and 5 mM lysine caused 96% inhibition.

RESPONSE TO ENERGY CHARGE. The lysine-sensitive aspartokinase of *E. coli* responds sharply to variation in energy charge (Figure 1). The response is in the direction to be expected for enzymes that participate in the regulation of biosynthetic pathways (Atkinson, 1968). In the absence of lysine the reaction velocity at 0.93 charge is 50% of the maximal velocity attained at full charge.

On the addition of lysine at increasing concentrations, the response to energy charge is progressively diminished (Figure 1). This result is consistent with the expectation that the activity of lysine-sensitive aspartokinase should be a joint function of the lysine concen-

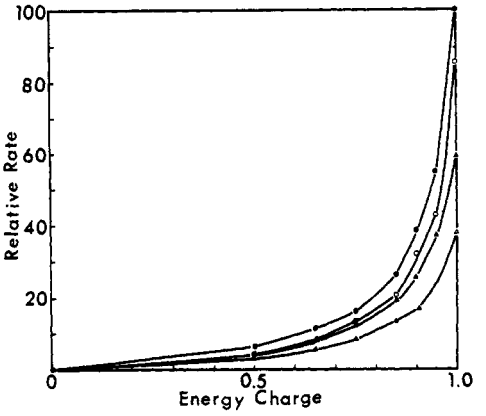

FIGURE 2: Rate of the reaction catalyzed by *E. coli* lysine-sensitive aspartokinase as a function of energy charge; interaction with concentration of NH_4^+. The experiments were performed as described in the legend for Figure 1, except that lysine was omitted and the amount of NH_4Cl varied between 0.8 and 20 μmoles. Concentration of NH_4Cl: (●) 20.0, (○) 6.0, (▲) 2.0, and (△) 0.8 mM.

tration and the energy balance of the cell. Interaction between energy charge and the concentration of NH_4^+ is shown in Figure 2.

PR-ATP Synthetase. KINETIC CONSTANTS. Under the conditions of the standard assay for PR-ATP synthetase one product, inorganic pyrophosphate, is removed by the action of pyrophosphatase. The other product, PR-ATP, accumulates and appears to inhibit synthetase activity considerably, even at the low concentrations reached in the assay mixture. It is therefore important

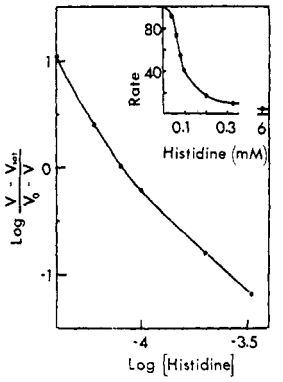

FIGURE 3: Rate of the reaction catalyzed by PR-ATP synthetase from *E. coli* strain X-9 as a function of histidine concentration. The main graph is a Hill plot of the data plotted with linear coordinates in the insert. The reaction mixture (0.3 ml) contained: 30 μmoles of Tris-HCl (pH 8.5), 45 μmoles of KCl, about 15 units (μmoles/min) of yeast pyrophosphatase, 0.75 μmole of ATP, 0.075 μmole of AMP, 1.5 μmoles of $MgCl_2$, 0.18 mg (protein) of the enzyme preparation containing about 0.1 unit of activity, and histidine as indicated. After a straight base-line recording was obtained, the reaction was started by the addition of 10 μl of 3.75 mM PRPP on a small plastic spatula (final PRPP concentration, 125 μM). The mixture was stirred quickly and the initial reaction rate was determined. In the Hill plot, concentration is expressed in molarity, and the symbols used are: v, velocity; v_0, velocity in the absence of histidine; v_{sat}, velocity in the presence of a saturating level of histidine.

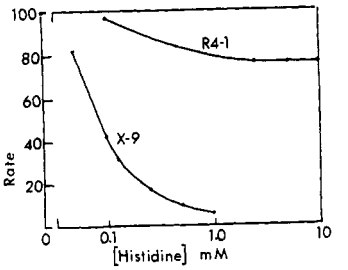

FIGURE 4: Effect of histidine on the rate of the reactions catalyzed by PR-ATP synthetases from *E. coli* strain X-9 and from thiazolealanine-resistant strain R4-1. The total concentration of adenine nucleotides was 2.5 mM, at an energy charge of 0.9 and equilibrated in the presence of adenylate kinase before initiation of the assay reaction. Enzyme protein levels were: X-9 (specific activity 0.1), 0.10 mg; R4-1 (specific activity 0.3), 0.02 mg. The assay was performed as described for Figure 3.

to determine the true initial rate of the reaction in studies of the behavior of this enzyme.

In the presence of 0.25 mM PRPP, the $(S)_{0.5}$ value for ATP was estimated at 30 μM. In the presence of 2 mM ATP, $(S)_{0.5}$ for PRPP could not be measured with reasonable accuracy by standard spectrophotometric techniques, but it appeared to be smaller than 5 μM.

RESPONSE TO ENERGY CHARGE AND HISTIDINE CONCENTRATION. Preparations of PR-ATP synthetase from *Salmonella typhimurium* and *E. coli* have been shown to be inhibited by histidine (Martin, 1963; Voll *et al.*, 1967; O'Donovan and Ingraham, 1965). The initial rate of the reaction catalyzed by our preparation from *E. coli* strain X-9 appeared to be relatively insensitive to histidine. During the course of the reaction, the degree of inhibition by histidine increased, suggesting the possibility of synergistic interaction between histidine and the immediate product of the reaction, PR-ATP. Inhibition by histidine was also enhanced by AMP, which inhibited only weakly when present alone.

The effect of histidine on the activity of PR-ATP synthetase from strain X-9 in the presence of AMP at 250 μM (one-tenth the ATP concentration) is shown in Figure 3. The sigmoid shape of the rate *vs.* histidine concentration curve (insert) suggests that histidine binds cooperatively. When corrected for the difference in scales of the coordinates, the slope of the Hill plot (main graph of Figure 3) ranges between about 3.5 and 1.8.

The sensitivities to histidine of the enzymes from strain X-9 and thiazolealanine-resistant strain R4-1 are compared in Figure 4. The enzyme from the resistant strain is inhibited much less strongly in the presence of saturating levels of histidine, and also requires more histidine for saturation.

The responses of the two enzymes to variation in energy charge are shown in Figures 5 and 6. In the absence of histidine, the response of the enzyme from strain X-9 (presumably identical with the synthetase from the parental wild-type strain) is quite different from that characteristic of other biosynthetic enzymes for which such curves have been obtained (Figure 5). On the addition of L-histidine at 0.1 mM, however, a curve of the

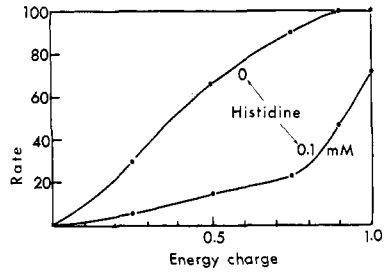

FIGURE 5: Rate of the reaction catalyzed by PR-ATP synthetase from *E. coli* strain X-9 as a function of energy charge; effect of histidine. A mixture of ATP and AMP, with the ATP mole fraction equal to the desired energy charge and at a total concentration of 2.5 mM, was incubated with adenylate kinase before the initiation of the assay, which was carried out as described for Figure 3.

usual type is obtained. It thus appears likely that in the intact cell this enzyme may respond sensitively to changes in either energy charge or the concentration of histidine. The enzyme preparation used for the experiments reported in Figure 5 was about 10-days old. Since sensitivity to histidine diminishes on storage, the histidine effect seen in Figure 5 should be considered minimal. All other PR-ATP synthetase results reported were obtained with freshly prepared enzyme. The behavior of the enzyme from thiazolealanine-resistant strain R4-1 is strikingly different (Figure 6), as would be expected from the responses of the two enzymes to histidine previously seen in Figure 4. Histidine at 0.1 mM, the level used in the experiments reported in Figure 5, had no significant effect on the enzyme from the resistant strain. Even when histidine was added at 100 times that concentration and appreciable inhibition resulted, the curve of rate as a function of energy charge was nearly linear at high charge values, rather than being concave upward in this region of interest. This pattern of response, like that seen in Figure 4, suggests that the enzyme from the resistant strain differs from the wild-type enzyme both in affinity for histidine and in the consequences of histidine binding.

Phosphoribosyl Pyrophosphate Synthetase. KINETIC CONSTANTS. Under standard assay conditions for PRPP synthetase, the observed value of $(S)_{0.5}$ was 0.2 mM for

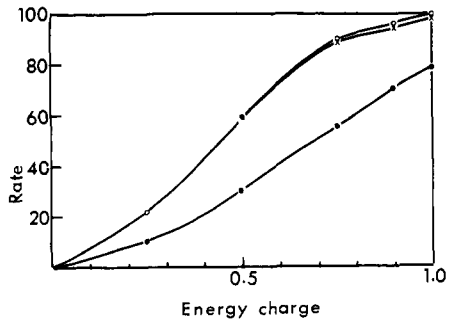

FIGURE 6: Rate of the reaction catalyzed by PR-ATP synthetase from *E. coli* strain R4-1 as a function of energy charge; effect of histidine. Conditions as described for Figure 5. Concentration of L-histidine: (○) 0, (×) 0.1, and (●) 10 mM.

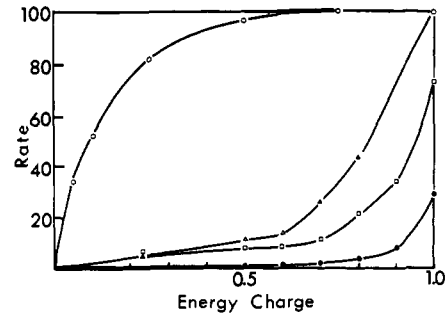

FIGURE 7: Rate of the reaction catalyzed by *E. coli* PRPP synthetase as a function of energy charge; effect of end products. A mixture of ATP and AMP, with the ATP mole fraction equal to the desired charge and at a total concentration of 1.8 mM, was incubated with 10 μg of adenylate kinase before addition of the other components of the reaction mixture: 90 μmoles of potassium phosphate (pH 7.5), 6 μmoles of $MgCl_2$, 0.5 μmole of ribose-5-P, and GDP, ITP, and UDP as indicated below. The assay volume was 1.1 ml. (○) ATP only; (△) charge response; (□) 1.0 mM each of GDP, ITP, and UDP; (●) 2.0 mM each of GDP, ITP, and UDP.

both ribose-5-P and ATP. Half-maximal velocity was observed when the $MgCl_2$ concentration was 0.5 mM. In Hill plots, the slopes of log $(v/(V_m - v))$ against log (S) varied between 1.0 and 1.3 for ribose 5-phosphate and between 1.5 and 1.8 for ATP. Hill slopes for $MgCl_2$ were consistently near 2.

RESPONSE TO ENERGY CHARGE AND END-PRODUCT CONCENTRATIONS. The catalytic activity of PRPP synthetase has been reported to respond to energy charge (Atkinson and Fall, 1967) and to be inhibited by purine and pyrimidine nucleotides and by tryptophan (Switzer, 1967; Atkinson and Fall, 1967). The interaction of these effects is shown in Figures 7 and 8. At energy charge values in the neighborhood of 0.9, the rate of the reaction was reduced by about 50% on the addition of three products (GDP, ITP, and UDP) at 1 mM each (Figure 7) or on the addition of eight products (UDP, UTP, IDP, ITP, GDP, GTP, dTTP, and tryptophan) at 0.3 mM each (Figure 8). This response seems to il-

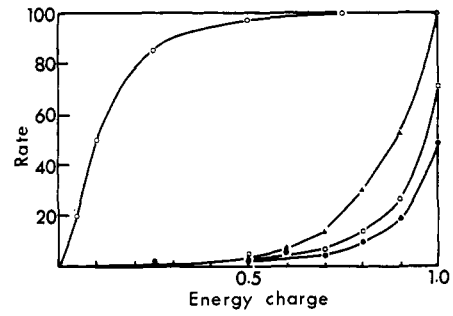

FIGURE 8: Rate of the reaction catalyzed by *E. coli* PRPP synthetase as a function of energy charge; effect of end products. Conditions as described for Figure 7, except that 60 μmoles of phosphate buffer was used, the total adenine nucleotide concentration was 2.0 mM, and the assay volume was 1.0 ml. The end products added were: UTP, UDP, ITP, IDP, GTP, GDP, dTTP, and tryptophan. (○) ATP only; (△) charge response; (□) 0.3 mM of each end product; (●) 0.6 mM of each end product.

lustrate interaction between energy charge modulation and cumulative product inhibition of the type first recognized in studies on glutamine synthetase (Woolfolk and Stadtman, 1964).

Discussion

Each of the three enzymes discussed in this paper catalyzes the first committed step in a biosynthetic sequence, and each responds to the energy charge of the adenylate system and to the concentration of one or more products of the sequence. The patterns of response are similar, and resemble that discussed generally in the preceding paper (Atkinson, 1968). If this *in vitro* pattern reflects the *in vivo* properties of the enzymes, it seems reasonable to suggest that at an energy charge of 0.85–0.90 and at physiological levels of end products, such enzymes may function at around 15–40% of capacity, and thus be in a position that allows sharp change of reaction velocity in either direction in response to changes in energy charge or product concentration.

Aspartokinase. The apparent Michaelis constants that we observed are much lower than those reported for the lysine-sensitive enzyme by Truffa-Bachi and Cohen (1966). A paper by Wampler and Westhead (1968), published while this manuscript was in preparation, reports values similar to ours. It seems possible that the high values initially reported may have resulted from the 800 mM NH_2OH present in the assay (but not in the assays used by Wampler and Westhead and by us).

The strong stimulation of aspartokinase by NH_4^+ suggests that the level of free NH_4^+ may be a factor in regulation of the production of the carbon chains required for amino acid synthesis. Such regulation of a pathway by a metabolite required for a later step is conceptually distinct from end-product feedback modulation, but the two types of control would obviously be compatible.

PR-ATP Synthetase. Our observed $(S)_{0.5}$ values for both ATP and PRPP are considerably lower than those previously reported for the enzyme from *Salmonella typhimurium* (Martin, 1963). This difference may reflect differences in the properties of enzymes from the two species, or, in view of the inhibition by PR-ATP, it may result from slight differences in assay procedures.

The pattern of response to energy charge and histidine concentrations, separately and together, is interesting. The enzyme is considerably more sensitive to histidine control in the presumed physiological range of energy charge than under standard assay conditions (Figure 5; 100 μM L-histidine caused about 62% inhibition at an energy charge of 0.85, but only about 28% at a charge of 1). Perhaps more important, the shape of the curve of response to energy charge is profoundly altered by the presence of histidine. This finding suggests that some enzyme-modifier interactions may be missed when potential modifiers are tested singly.

A high degree of stringency in the enzymic structure underlying the interaction between energy charge and histidine concentration is suggested by the observation that the enzyme from strain R4-1 not only has a much lower affinity for histidine, but has totally lost the synergistic interaction between charge and histidine concentration exhibited by the enzyme from the parental strain.

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