One way in which an organism controls its biosynthetic pathways is by feedback inhibition. This control adjusts the rate of production of the end products of pathways, such as amino acids and nucleotides, to the rate of synthesis of macromolecules, such as proteins and nucleic acids. Control is imposed by the end product itself in a simple and rapid manner: the end product stops production of more of itself by inhibiting the activity of the first enzyme unique to its pathway. Consequently, if an end product is not continually removed into macromolecules, additional end product is not made. The organism benefits from feedback inhibition, because it avoids wasting resources of carbon, nitrogen, and energy on end products which are not used. Certainly it is a widespread and effective metabolic control. Many biosynthetic pathways in bacteria have feedback inhibition, and a few instances are also known for higher organisms (1).

In this article, a feedback inhibition is investigated to learn more about its enzymological mechanism. Until now, feedback inhibition has been studied at a metabolic level where one usually asks which end product inhibits which enzyme. The question now asked is, does the end product of a pathway inhibit the first enzyme? This question is justified because many known feedback inhibitions appear competitive, as if inhibition results from a competition of the inhibitor and substrate for the same groups of the active site on the first enzyme. To the enzymologist, competition is wholly unexpected because the inhibitor results from a competition of the inhibitor and substrate, in contrast with classical competitors (2). How can there be competition when a site specific for the substrate seems unsuited for also binding the end product?

The problem of the mechanism of inhibition was approached by using the first enzyme unique to pyrimidine biosynthesis in Escherichia coli. This enzyme is aspartate transcarbamylase, which catalyzes the reaction shown in Fig. 1. It is known that in the bacterium the activity of aspartate transcarbamylase is closely controlled by an end product, probably a cytosine derivative, and that inhibition of this enzyme is important for controlling the whole pathway (3). Aspartate transcarbamylase is especially suited for enzymological study because it can be obtained easily in a highly purified state (4). It is inhibited by a nucleotide end product which competes with a structurally unrelated substrate, an amino acid.

Experiments to be described have two purposes: first, to find the structure required for an inhibitor, and, second, to find the structure required of the enzyme. It is proposed as a result of this work that the enzyme has special properties which make it sensitive to inhibition by the end product of the pathway. The enzyme has a second site, distinct from the active site, for which the end product has high affinity. The bound end product perhaps inhibits by deforming the enzyme so that the latter has a low affinity for the substrate.

EXPERIMENTAL PROCEDURE

Chemicals—All chemicals were obtained commercially except N-methylcytidine, which was a gift from Dr. J. J. Fox of the Sloan Kettering Institute, and carbamyl aspartic acid, which was synthesized by R. A. Yates according to the method of Nye and Mitchell (5).

Commercial dilithium carbamyl phosphate contained an impurity, probably urea (6), which interfered with the colorimetric assay. It was freed from the impurity by precipitation from an ice-cold solution (25 mg per ml) into which was stirred an equal volume of ice-cold 95% ethanol. After standing 30 minutes at 0°, the precipitate was collected and washed with ice-cold 95% ethanol on a Büchner funnel. It was then broken up and dried over concentrated H2SO4 in a desiccator at 0°. The product contained approximately 75% dilithium carbamyl phosphate by weight, as assayed by its enzymatic conversion to carbamyl aspartate. The remaining 25% of the material, probably lithium phosphate and firmly bound H2O (6), did not have any obvious stimulatory or toxic effect on aspartate transcarbamylase, since the dependence of reaction rate on carbamyl phosphate concentration was not unusual.

Enzyme Assay—Aspartate transcarbamylase was assayed by carbamyl aspartate production. One unit of enzymatic activity produces 1 μmole of carbamyl aspartate per hour. Standard assay conditions for the enzyme in extracts have been described previously (4). These conditions were varied as follows for studies with the highly purified enzyme. Tubes are prepared to contain 0.05 ml of fresh dilithium carbamyl phosphate solution (kept at 0° after solid is dissolved, and used within 2 hours), 0.05 ml of aspartic acid solution neutralized with KOH, 0.02 ml of buffer, and 0.33 ml of H2O. Reagent concentrations and pH values for each experiment are given in the figures and tables. The tubes are equilibrated at 28° for 5 minutes, and the reaction is begun by adding 0.05 ml of a solution of aspartate transcarbamylase in 5 × 10⁻³ m potassium phosphate buffer, pH 7.0, kept at 0°. The total reaction volume is 0.50 ml. The amount of enzyme is chosen to produce approximately 0.10 μmole of carbamyl aspartate during the incubation. After 30 minutes, the reaction is stopped by the addition of 2.5 ml of the 3:1:1
mixture described below. Tubes are put on ice until the colorimetric analysis for carbamyl aspartate is performed.

**Colorimetric Determination of Carbamyl Aspartate—**In our hands, the original Koritz and Cohen procedure for carbamyl amino acids (7) was not sensitive or reproducible enough to use for accurate enzyme kinetics. It has been modified in the past by Crokaert and Schram (8), and the method described here incorporates some of their conditions. Temperatures and concentrations are determined specifically for carbamyl aspartate; other carbamyl derivatives probably require slightly different conditions for maximal sensitivity. By this method, a quantity of 0.1 µmole of carbamyl aspartate gives a final optical density of 0.70 at 560 nm (1-cm light path). This represents an 8-fold increase in sensitivity over the Koritz and Cohen procedure and a 4-fold increase over the Crokaert and Schram modification. Readings are reproducible within 3% from day to day if time intervals between steps are standardized.

Stock reagents for this procedure are (a) 66 ml of concentrated H2SO4 added to 34 ml of H2O at 0°; (b) 22.5 mg of diacetylmonoxime (2,3 butanedione-2-oxime) per ml of water, kept at 0° and protected from light; (c) diphenylamine-p-sulfonate solution containing 114 mg of Na2 diphenylamine-p-sulfonate dissolved in 100 ml of 0.1 N HCl which contains 0.4 g of Atlas BRIJ 35, kept at 0° and protected from light; and (d) 2.5 mg of K2S2O8 per ml of water, kept at 0°.

Just before determination, reagents (a), (b), and (c) are mixed at 0° in the ratio 3:1:1 by volume; 2.50 ml of 3:1:1 mixture are added to 0.50 ml of enzyme reaction mixture, shaken, and kept at 0° until proceeding further. Tubes are then heated at 60° for 30 minutes and cooled in ice water. They are kept at 0° until the next step, in which they are equilibrated at 28° for several minutes before the addition of K2S2O8 solution (0.50 ml) with shaking. Optical density is read at 560 nm when maximal color has developed, usually approximately 20 minutes after the addition of K2S2O8. The color then fades at a rate of approximately 1% per minute. Addition of 0.5 ml of dioxane and lower temperatures greatly decrease the fading rate, but generally such precautions have not been taken. If the tubes are allowed to stand at 0° for several hours before or after heating, slightly more color is finally produced.

**Purification of Aspartate Transcarbamylase—**A procedure has already been reported for obtaining aspartate transcarbamylase in a physically homogeneous state (4). It was modified slightly in order to obtain a preparation of enzyme highly sensitive to inhibition by cytidine derivatives. Most important, all solutions coming in contact with the enzyme contained 0.001 M mercaptoethanol and 0.0001 M neutral EDTA, since the inhibitor site of the enzyme is sensitive to heavy metal ions. In the purification procedure, the major change was omission of the (NH4)2SO4 precipitation step. Instead, after the supernatant fluid from the heated extract was neutralized with 2 N NH4OH and made 0.01 M in imidazole hydrochloride buffer, pH 7.0, the diethylaminoethyl (DEAE) cellulose fractionation step was carried out directly. The enzyme was eluted at 0.15 M KCl, a slightly higher KCl concentration than reported previously.

After collection of the enzyme on a 0.6-g column as described for the concentrating step, it was subjected to elution in 0.01-M steps of 20 ml each from 0.11 M KCl to 0.18 M KCl. Again the enzyme was eluted at approximately 0.15 M KCl. It was then concentrated to a volume of approximately 4 ml.

Finally, the KCl was removed, and the buffer was changed by passing the enzyme through a column of Sephadex G-50 equilibrated with 0.020 M potassium phosphate buffer, pH 7.0, 0.002 M mercaptoethanol, and 0.0002 M neutral EDTA. After storage for 3 months at 4° in this solution, the enzyme had lost no activity or sensitivity to inhibitors. The specific activity (4) of this highly purified preparation was 4500 units per mg of protein. The enzyme of this preparation was very sensitive to feedback inhibitors. It could be maximally inhibited by CTP to approximately 85 to 90%. Possible explanations for this incomplete inhibition will be discussed later. By the criterion of ultracentrifugation, the preparation had one major component, indicating a high degree of homogeneity. The sedimentation coefficient was equivalent to the value reported earlier for aspartate transcarbamylase (4).

**RESULTS**

**Inhibition by Cytosine Derivatives—**In the earlier studies with crude extracts (3), it seemed that the observed inhibition by cytosine derivatives might be indirect and require conversion of the added compound to some inhibitory form. Indeed, it has been found that partially purified aspartate transcarbamylase was insensitive to cytosine derivatives (1).

Highly purified aspartate transcarbamylase was, however, inhibited by cytosine derivatives. Of the compounds tested, CTP and dCTP were the strongest inhibitors. The enzyme was inhibited more than 70% by 10⁻⁴ M CTP or dCTP when the aspartate concentration was below 2.5 X 10⁻⁴ M. The inhibition was specific for cytosine nucleosides and nucleotides; other pyrimidine- and purine-containing compounds inhibited weakly, if at all (Table I). For example, the 35% inhibition by GTP was equaled by 0.02 the amount of CTP. This finding of inhibition with purified aspartate transcarbamylase shows that cytosine derivatives themselves act directly on the enzyme.

The data obtained with cytosine derivatives (Table I) show that the entire CTP molecule functions in inhibition. Cytosine alone did not inhibit; but cytidine and deoxyctydine, which contain a sugar, did inhibit weakly. Each phosphate added to the 5' position of the nucleoside, as in CMP, CDP, and finally CTP, resulted in a decided increase in inhibition. Therefore, the sugar and three phosphates all contributed significantly to the binding of CTP to the enzyme.

The presence of cytosine was an absolute requirement for strong inhibition. For example, when the free amino group of cytosine was changed to a hydroxyl as in UTP, or blocked as in

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1. P. Reichard, personal communication.
the methylamino nucleoside, inhibition was lost. The substitution of purines at the base position of nucleotides had profound effects on inhibitory strength. ATP was a stimulator of activity (to be discussed later), and GTP was a weak inhibitor. The weak inhibition by GTP was due to more than simply poor binding to the enzyme. As shown in Table II, GTP inhibited maximally only approximately 40 to 50%, as if, in this case, the enzyme inhibitor complex was still enzymatically active.

Kinetics of Inhibition—Once the strength and specificity of CTP inhibition were established, the kinetics of inhibition was studied. It was first determined that CTP, as well as other cytosine derivatives, appeared to inhibit the enzyme competitively. CTP inhibition could be reversed by high concentrations of one of the substrates, aspartate (Fig. 2). Despite the complex kinetics, it is apparent that 70% inhibition at 5 × 10⁻³ M aspartate was reduced to less than 5% inhibition by 25 × 10⁻³ M aspartate. CTP inhibition formally resembled other competitive inhibitions in two major respects: first, the inhibitor increased the concentration of substrate required for half saturation; second, the inhibitor did not influence the maximal velocity.

Earlier it was reported that 5'-CMP competed with the other substrate, carbamyl phosphate (3). The present tests do not support this result; carbamyl phosphate did not influence the inhibition by CMP or CTP. No explanation can be given for the discrepancy. The earlier work was done with crude extracts of low activity, requiring long incubations. The earlier work was done with E. coli strain B and this work was done with K-12; but in crude extracts of both strains, CMP competed with aspartate and not with carbamyl phosphate.

Selective Destruction of Feedback Inhibition—CTP probably does not attach to the aspartate binding site, although it appears to compete with aspartate. That site is very specific; neither ρ-methyl aspartate (threo or erythro) nor L-glutamate interacted with it. CTP probably binds to an entirely different site, one suitable for cytosine, a sugar, and three phosphates. If the inhibitor-binding site is really specific and has little to do with binding the substrate, then disrupting this site should greatly reduce the inhibition, but should not necessarily affect the enzymatic activity. Indeed, the enzyme might be treated so that it becomes insensitive to the inhibitor yet is still active. If, however, both substrate and inhibitor depended on the same site for binding, this separation would not be possible.

Three ways have been found for destroying inhibition completely while leaving the enzyme fully active (Table III). One was to heat the enzyme at 60° in 5 × 10⁻³ M potassium phosphate buffer, pH 7.0. The enzyme could no longer be inhibited after 3 minutes of this treatment. Activity was not lost, even after 20 minutes, but rather increased 2-fold. Mercaptoethanol

TABLE I

Inhibition of aspartate transcarbamylase by pyrimidine and purine derivatives

The reaction mixture contained 3.6 × 10⁻³ M carbamyl phosphate, 5 × 10⁻³ M aspartate (unsaturating), 2.0 × 10⁻³ M inhibitor (neutralized), 0.04 M potassium phosphate buffer, pH 7.0, and 2.2 × 10⁻¹ μg of protein per ml.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition by 0.002 M compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cytosine</td>
<td>%</td>
</tr>
<tr>
<td>2. Cytidine</td>
<td>0</td>
</tr>
<tr>
<td>3. Deoxyxystydine</td>
<td>22</td>
</tr>
<tr>
<td>4. CMP</td>
<td>38</td>
</tr>
<tr>
<td>5. dCMP</td>
<td>48</td>
</tr>
<tr>
<td>6. CDP</td>
<td>68</td>
</tr>
<tr>
<td>7. CTP</td>
<td>88</td>
</tr>
<tr>
<td>8. dCTP</td>
<td>88</td>
</tr>
<tr>
<td>9. UTP</td>
<td>8</td>
</tr>
<tr>
<td>10. dUTP</td>
<td>23</td>
</tr>
<tr>
<td>11. 2',3' mixed CMP</td>
<td>24</td>
</tr>
<tr>
<td>12. N-methylytidine</td>
<td>0</td>
</tr>
<tr>
<td>13. ATP</td>
<td>-180*</td>
</tr>
<tr>
<td>14. dATP</td>
<td>-162*</td>
</tr>
</tbody>
</table>

* Stimulation.

TABLE II

Incomplete inhibition by GTP and CTP

The reaction mixtures were prepared as described for Table I, except that the inhibitor concentration was varied as indicated.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTP [μM]</td>
<td>CTP [μM]</td>
</tr>
<tr>
<td>4 × 10⁻⁴</td>
<td>15</td>
</tr>
<tr>
<td>1 × 10⁻³</td>
<td>25</td>
</tr>
<tr>
<td>2 × 10⁻³</td>
<td>35</td>
</tr>
<tr>
<td>4 × 10⁻³</td>
<td>40</td>
</tr>
<tr>
<td>2.5 × 10⁻⁵</td>
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</tr>
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<td>5 × 10⁻⁴</td>
<td>44</td>
</tr>
<tr>
<td>5 × 10⁻³</td>
<td>74</td>
</tr>
<tr>
<td>2 × 10⁻³</td>
<td>85</td>
</tr>
</tbody>
</table>

FIG. 2. Reversal of CTP inhibition by aspartate. Velocity = units of activity per mg of protein × 10⁻⁵. The reaction mixture contained 3.6 × 10⁻³ M carbamyl phosphate, aspartate varied as indicated; 2.0 × 10⁻³ M CTP when used; 0.04 M potassium phosphate buffer, pH 7.0, and 9.0 × 10⁻⁴ μg of enzyme protein per ml.

2 Further kinetics studies and possible explanations for their anomalous appearance may be found in the thesis of J. C. Gerhart, University of California, 1962.
The reaction mixture contained 3.6 X 10^-2 M carbamyl phosphate, 5.0 X 10^-4 M aspartate, 0.04 M potassium phosphate buffer, pH 7.0, 1.8 X 10^-4 M of enzyme protein per ml, and heavy metal compounds or urea as indicated in this table. Sensitivity to CTP inhibition was destroyed by heating as follows. The enzyme was diluted to 1.8 M protein per ml in 5 X 10^-3 M potassium phosphate, pH 7.0, and put in a 60° water bath for 4 minutes, after which it was cooled quickly in ice water.

The second method for selectively destroying the inhibition was treatment with urea. In the presence of 0.8 M urea, aspartate transcarbamylase could not be inhibited at all by CTP, yet activity was unchanged. Heating and urea seem to denature the enzyme locally at the inhibitor site. This site seems to be more delicate in structure than the part of the enzyme bearing the active site.

The third method which produced a selective inactivation was treatment with Hg^2+, mercurials, or Ag^+. The effect was rapid; a few minutes at most were required. Some group or groups of the enzyme, perhaps sulfhydryl, were attacked by the heavy metal, but it cannot be said that this alone caused the loss of sensitivity to CTP. Other sulfhydryl agents such as iodoacetate, arsenate, Zn^2+, and Cd^2+ had no effect on either inhibition or activity. The loss was not reversible. Perhaps a change in protein structure followed the heavy metal attack.

The selective destruction of inhibition provides evidence that the site for binding the inhibitor is largely independent of the site for binding the substrates. However, the two sites are not entirely independent; disruption of the inhibitor site did affect the active site profoundly. It was found that the kinetics of the enzyme treated with mercurials or heat was markedly different from those of the native enzyme in three ways. First, the maximal velocity doubled; second, the concentration at which the enzyme was half saturated by aspartate doubled (12 X 10^-3 M); and third, the dependence of velocity on aspartate concentration followed a curve unlike the unusual sigmoidal dependence of the native enzyme (Fig. 3). It was also found that the pH optimum (measured at 5 X 10^-3 M aspartate) changed from 7 to 8.5. The effect of heating or mercurials on activity was most favorably observed at pH 8.5 in the presence of 5 X 10^-3 M aspartate; a 15-fold increase in activity accompanied the destruction of the inhibitor site. The sedimentation coefficient (s_20) decreased from 11.6 S to 5.9 S after heating.

**Fig. 3.** Dependence of rate on aspartate after loss of feedback inhibition. Velocity = units of activity per mg of protein X 10^-3. * = native (untreated) aspartate transcarbamylase; 10^-4 M HgNO_3 present during assay. The reaction mixture contained 3.6 X 10^-2 M carbamyl phosphate; aspartate varied as indicated. The second method for selectively destroying the inhibition was treatment with urea. In the presence of 0.8 M urea, aspartate transcarbamylase could not be inhibited at all by CTP, yet activity was unchanged. Heating and urea seem to denote the enzyme locally at the inhibitor site. This site seems to be more delicate in structure than the part of the enzyme bearing the active site.

**Fig. 4.** Reversal of CTP inhibition by ATP. Velocity = units of activity per mg of protein divided by 20. The velocity curve refers to the stimulation of the enzyme by ATP. The extent to which stimulation was reduced by 10^-4 M CTP is described by the percentage inhibition curve. The reaction mixture contained 3.6 X 10^-2 M carbamyl phosphate; 2.5 X 10^-3 M aspartate; ATP varied as indicated. 1.0 X 10^-4 M CTP when used; 0.04 M potassium phosphate, pH 7.0; and 4.5 X 10^-2 M of protein per ml.
nucleotides, it is likely that ATP and CTP bind largely at the same site on this enzyme.

ATP stimulated enzyme activity even in the absence of CTP (Fig. 4). $V_{\text{max}}$ was not changed, but the concentration of aspartate required for half-saturation of the enzyme was lowered (Fig. 5). Treatment with mercurials or heat rendered the enzyme insensitive to ATP as well as to CTP. This simultaneous loss of both nucleotide effects would be expected if a single and common site was disrupted. The site would be specific for nucleotides and distinct from the active site.

**DISCUSSION**

From this work a model can be described for the surface of a feedback-inhibited enzyme, aspartate transcarbamylase. The enzyme has groups in its active site for binding its substrates, aspartate and carbamyl phosphate, as do other enzymes. The novel point is that aspartate transcarbamylase has additional groups for binding its feedback inhibitor, CTP. Some, and perhaps all, of these groups are not in the active site, but compose instead what will be called the "feedback site." This site is concerned with the function of control. The model, which may apply to other feedback-inhibited enzymes, answers several enzymological questions. Briefly, it accounts for the way in which an enzyme binds a substrate and an inhibitor which differ in size, shape, and charge. Also, for transcarbamylase it indicates how an enzyme loses sensitivity to an inhibitor without losing catalytic activity at the same time.

This two-site model describes only the binding of a feedback inhibitor. The question of how CTP inhibits if it is not bound at the active site remains. The existence of a second site makes it necessary to entertain many more mechanisms of inhibition that would be applicable in the case of a single site. The favored mechanism assumes that CTP and aspartate can occupy the enzyme simultaneously, each at its own site. Also, it assumes that the enzyme-inhibitor complex has full catalytic activity. According to this mechanism, CTP inhibits by greatly reducing, but not abolishing, the affinity of the enzyme for aspartate. In short, when CTP occupies an enzyme molecule, the $K_m$ or concentration of substrate for half-saturation, for aspartate is increased for that molecule, but its $V_{\text{max}}$ is unchanged. This mechanism, as well as many others (9), is consistent with the observed reversal of CTP inhibition by aspartate.

The evidence in favor of this mechanism is the finding that aspartate transcarbamylase is still active when saturated with nucleotides. Thus, when aspartate concentration was $5 \times 10^{-3}$ M, CTP inhibited maximally approximately 55 to 90%. ATP inhibited maximally approximately 40 to 50%, and CTP stimulated maximally approximately 180%. It is thought that all three nucleotides bind at the feedback site and bring about their quantitatively different effects by a common mechanism of altering the enzyme's affinity for aspartate.

Combination of CTP at the feedback site might reshape the enzyme so that the active site is slightly distorted. Strong interactions between the two sites appear to exist even in the absence of CTP, as expressed by the marked changes in enzymatic activity accompanying the loss of sensitivity to CTP. Thus, the heated enzyme was 15 times as active as the native enzyme under certain assay conditions. This increase reflects changes in $V_{\text{max}}$, substrate affinity, and pH optimum, indicating an extensive rearrangement of the protein. The sedimentation coefficient ($s_{20}$) was 11.6 S before heating and 5.9 S after heating. The greatly changed sedimentation coefficient provides direct evidence for such a change in protein structure.

The existence of a feedback site accounts for the surprising specificity with which feedback inhibition operates in an organism. This specificity exhibits itself in two ways. First, the end product alone inhibits the first enzyme of its pathway. Second, inhibition is of a strength such that the amount of end product in a cell is not growth-limiting at the one extreme, or wasteful at the other. Feedback inhibition is not to be viewed as a fortunate coincidence of structures of end products, first step enzymes, and substrates. It is an effective control because the first enzyme of a biosynthetic pathway is specially constructed to be feedback-inhibited. The enzyme has on its surface a "feedback site" built for binding the end product, no matter how much the end product differs from the substrate. Enzymes for other steps of cellular metabolism probably do not have such a site. It is only in instances of feedback inhibition that structurally unrelated metabolites interfere with each other to the advantage of the organism.

An extrapolation to the operation of feedback inhibition in the pyrimidine pathway of intact organisms can be made from the results of this work. Cytosine nucleotides such as UDP, CTP, and dCTP were by far the best inhibitors of highly purified aspartate transcarbamylase among the pyrimidine and purine derivatives tested. If this specificity may be extrapolated to intact E. coli, it is likely that CTP alone largely controls the activity of the pathway. CTP is more abundant in the growing bacterium than other cytosine derivatives; its concentration has been estimated at $10^{-4}$ M (10). This concentration of CTP inhibited purified transcarbamylase strongly, more than 70%, when aspartate was below approximately $2 \times 10^{-3}$ M, as it may be in the cell.

It could be argued that the residual activity was due to a fraction of the preparation which was not sensitive to CTP. Crude extracts were tested, and there, also, the enzyme could not be inhibited completely. Possibly the bacterial cell produces a second enzyme, or aspartate transcarbamylase is unstable enough that a few molecules always lose sensitivity to CTP. Such a fraction would have to follow aspartate transcarbamylase throughout the purification or be continually replenished.

![Fig. 5. Effect of ATP on reaction velocity. Velocity = units of activity per mg of protein x $10^{-1}$. The reaction mixture contained $3.6 \times 10^{-3}$ M carbamyl phosphate; aspartate varied as indicated; $2 \times 10^{-3}$ M ATP when used; $0.04$ M potassium phosphate buffer, pH 7.0; and $9.0 \times 10^{-2}$ µg of enzyme protein per ml.](http://www.jbc.org/content/237/3/895.f1)
The physiological importance of ATP stimulation is uncertain. The ATP concentration in the cell (10) is sufficient to cause definite stimulation of activity and slight reversal of CTP inhibition, if the response of aspartate transcarbamylase is the same as it was in extracts. If this stimulation occurs in the bacterium, its effect would be to speed up pyrimidine biosynthesis when the concentration of purines is high. Thus, the pyrimidine pool would increase in step with an increase in the purine pool. Presumably, the synthesis of both purines and pyrimidines must accelerate before the synthesis of nucleic acid can accelerate.

Feedback inhibition takes on the aspect of a carefully evolved phenomenon. The evolutionary sequence of events for aspartate transcarbamylase and other similar enzymes may have been as follows. At first, the transcarbamylase may have merely catalyzed its reaction; it bound its substrates, but not the end product, which was a very different molecule. Its enzymatic activity was not controlled, as with certain mutants which lack feedback inhibition in the histidine and tryptophan pathways (11, 12). Later, through a series of mutations and selections for organisms making most efficient use of their nutrients, aspartate transcarbamylase could have gained a special site which bound the end product in such a way that the inhibitor impaired the substrate's attachment. Organisms making this modified enzyme could control pyrimidine biosynthesis so as to have a selective advantage.

SUMMARY

1. The inhibition of aspartate transcarbamylase was studied with highly purified enzyme from Escherichia coli. The enzyme was strongly inhibited by cytidine triphosphate, the probable feedback inhibitor in the bacterium.

2. Cytidine triphosphate inhibition was reversed by high concentrations of aspartate, one of the substrates. In the presence of cytidine triphosphate, the concentration at which aspartate half saturated the enzyme increased, but maximal velocity remained unchanged.

3. The entire cytidine triphosphate molecule functioned in inhibition. In decreasing inhibitory strength were cytidine triphosphate, cytidine diphosphate, cytidine 5'-phosphate, cytidine, and cytosine.

4. Aspartate transcarbamylase was altered by heat, urea, or heavy metal ions so that it was no longer sensitive to cytidine triphosphate inhibition. At the same time, enzymatic activity increased 15-fold at pH 8.5. The pH optimum, maximal velocity, and concentration at which aspartate half saturated the enzyme changed markedly.

5. It is concluded that the surface of aspartate transcarbamylase contains an exclusive site for binding cytidine triphosphate, the feedback inhibitor. This feedback site is distinct from the active site but appears to influence the function of the latter.

6. Enzymes probably gained feedback sites during the course of evolution, since mutant bacteria possessing these sites would have a growth advantage.

REFERENCES

The Enzymology of Control by Feedback Inhibition
John C. Gerhart and Arthur B. Pardee


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