Anthranilate Synthetase, an Enzyme Specified by the Tryptophan Operon of *Escherichia coli*: Comparative Studies on the Complex and the Subunits

JUNETSU ITO and CHARLES YANOFSKY

*Institute for Microbial Diseases, Osaka University, Osaka, Japan, and Department of Biological Sciences, Stanford University, Stanford, California 94305*

Received for publication 31 July 1968

The properties of the anthranilate synthetase complex and its separated subunits were compared in catalyzing the anthranilate synthetase reaction, chorismate + L-glutamine or NH₄⁺ → anthranilate, and the transferase reaction, anthranilate + 5'-phosphoribosyl-1-pyrophosphate → phosphoribosyl anthranilate. It is shown that anthranilate synthetase component I is activated by normal anthranilate synthetase component II, a component ICRM (CRM = immunologically cross-reacting material), and by a presumed fragment of component II produced by a deletion mutant. Significant differences between the complex and its subunits are demonstrated with respect to substrate affinity, thermostability, feedback inhibitor sensitivity, and activity in the presence of various divalent cations. Of particular interest are the findings that the transferase activity of component II is only inhibitable by L-tryptophan when the component is in the complex and that this inhibition does not appear to depend upon the feedback-sensitive site of component I.

In the preceding paper (10), we described the purification and properties of anthranilate synthetase component I (CoI), the product of the *trpE* gene of the tryptophan operon of *Escherichia coli*. The present paper deals with the activation of anthranilate synthetase CoI by anthranilate synthetase component II (CoII), CoICRM (CRM = immunologically cross-reacting material), and a fragment of CoII. Anthranilate synthetase CoII is specified by the *trpD* gene of the tryptophan operon. The properties of the anthranilate synthetase complex and the separate components are compared.

Similar studies have been carried out with the *Salmonella typhimurium* enzyme and its components by Bauerle and Margolin (2).

**MATERIALS AND METHODS**

**Organisms.** Auxotrophs of the K-12 strain of *Escherichia coli* were employed in this study. Most of the mutants were isolated by penicillin selection after ultraviolet irradiation of prototrophic strains, and were described previously (12, 20, 21). The strain carrying *trpD*7775 is a D gene nonsense (ochre) mutant, and *trpE*3 (previous designation, T3) is an E gene missense mutant. Strains carrying *trpD*7778 and *trpE*572 have been used, respectively, as sources of the two anthranilate synthetase components, CoI and CoII. Mutant *trpA*4 is a deletion of the A, B, and C genes and part of the D gene of the tryptophan operon. A strain carrying *trpA*4 is a double mutant with a deletion of genes A, B, and C and a part of D, which forms feedback-resistant (tryptophan-insensitive) anthranilate synthetase CoI. Mutant A2/C2 is an episome-bearing strain which forms high levels of the wild-type anthranilate synthetase complex (1). This strain was kindly provided by J. P. Crawford.

**Preparation of cell extracts.** Cells were cultured in minimal medium supplemented with 0.4% glucose and 5 μg of L-tryptophan per ml. A supplement of 0.01% acid-hydrolyzed casein was included in the growth medium of the mutant carrying the deletion *trpA*4 to increase the yield of bacteria. Cells were harvested by centrifugation, washed once with 0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer at pH 7.8, and resuspended in the same buffer. After disruption by sonic treatment, cell debris was removed from the suspensions by centrifugation at 30,000 × g for 30 min.

**Enzyme fractionation.** Unless otherwise indicated, partially purified preparations of the anthranilate synthetase complex were used in this study. The complex was purified by the procedure of Baker and 

---

1 Present address: Institute for Microbial Diseases, Osaka University, Osaka, Japan.
Crawford (1) with minor modifications. Purification involved ammonium sulfate fractionation, Sephadex G-200 gel filtration, diethylaminoethyl (DEAE)-Sephadex column chromatography, and zonal centrifugation in a sucrose gradient.

Anthraniolate synthetase CoI was purified from extracts of the strain carrying trpD9778, as described in the accompanying paper (10). Anthranilate synthetase CoII (phosphoribosyl anthranilate (PR) transferase) was partially purified as follows. To 400 ml of a crude extract of the strain carrying trpE972, 94 g of solid ammonium sulfate was added, and the precipitate was collected by centrifugation. The precipitate was dissolved in a small amount of 0.1 M Tris-hydrochloride buffer (pH 7.8) containing 0.1 M β-mercaptoethanol and dialyzed against the same buffer for 4 hr in the cold. The dialysate was applied to the top of a Sephadex G-100 column (3 by 60 cm) equilibrated with 0.1 M Tris-hydrochloride buffer (pH 7.8). The column was eluted with the same buffer. Fractions with appreciable activity were combined and the protein was precipitated by the addition of solid ammonium sulfate (28.8 g/100 ml). The resulting precipitate was collected by centrifugation and suspended in a small amount of 0.1 M Tris-hydrochloride buffer and dialyzed against 0.05 M Tris-hydrochloride buffer overnight with one change of buffer. The dialysate was placed on a column of DEAE-Sephadex A-50 (2 by 50 cm) equilibrated with the same buffer. Protein was eluted with a linear gradient of 0.05 M (1 liter) to 0.7 M (1 liter) Tris-hydrochloride buffer at pH 7.8. A purification of approximately 25-fold (compared to the crude extract) was obtained by the above procedure.

Enzyme assays. The activity of the anthranilate synthetase complex was assayed as described previously (12, 21). The assay procedures for anthranilate synthetase CoI and CoII are described in the accompanying paper (10). Anthranilate synthetase CoII was assayed for PR transferase activity as follows. A reaction mixture was prepared containing 10⁻⁴ M anthranilate, 10⁻⁴ M 5'-phosphorylribosyl-1-pyrophosphate (PRPP), 4 × 10⁻⁴ M magnesium acetate, 2 × 10⁻⁴ M β-mercaptoethanol, 5 × 10⁻³ M Tris-hydrochloride buffer at pH 7.8, enzyme solution (usually 0.02 to 0.05 ml), and distilled water in a final volume of 2 ml. The reaction was followed at 37 C by observing the decrease in fluorescence at 396 nm (activation wavelength, 315 nm) in an Amino-Bowman recording spectrophotofluorometer. In later studies, an Hitachi recording spectrophotometer was employed. One unit of anthranilate synthetase CoII is defined as the amount of enzyme required to consume 0.1 μmole of anthranilate in 20 min at 37 C. Specific activity is expressed as units of enzyme per milligram of protein. Protein was determined by the method of Lowry et al. (15).

Catalase activity was assayed as described by Martin and Ames (16). Bacterial alkaline phosphatase was determined by the method of Schlesinger and Barrett (18).

Chemicals. PRPP (magnesium salt) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. d-Tryptophan was kindly supplied by Tanabe Co. Chorismic acid was prepared as described elsewhere (9, 10). Other chemicals were reagent grade.

RESULTS

Effect of anthranilate synthetase CoI and CoII concentration on anthranilate synthetase activity. When glutamine rather than NH₄⁺ is employed as substrate in the anthranilate synthetase reaction, purified anthranilate synthetase CoI is inactive unless anthranilate synthetase CoII is also present. Thus, when glutamine is the substrate, it is possible to examine the dependence of enzyme activity on the relative amounts of the two components.

Figure 1 shows how anthranilate synthetase activity varies as a function of the concentration of anthranilate synthetase CoII, when excess anthranilate synthetase CoI is present. Anthranilate synthetase activity is obviously dependent upon the presence of component II, and increases linearly with increasing amounts of this component. A similar result was obtained when anthranilate synthetase CoI concentration was varied in the presence of excess anthranilate synthetase CoII. Also, one unit of PR-transferase is very nearly equivalent to one unit of component II (Fig. 1), i.e., transferase units added

![Units of PRTase added](http://jb.asm.org/)
were almost identical with anthranilate synthetase units detected.

Activation of anthranilate synthetase CoI by an altered anthranilate synthetase CoII. Accumulation studies with D gene mutants permit the recognition of two distinct classes; one class does not accumulate anthranilic acid in its culture medium, whereas the other class does. The first class is composed of nonsense mutants which produce unassociated anthranilate synthetase CoI. Since members of the second class accumulate anthranilic acid, it was suspected that they form an altered component II which complexes with, and activates, component I. To examine this possibility, a crude extract of the strain carrying the D gene missense mutation trpD80 was prepared and was analyzed by sucrose density-gradient centrifugation. Figure 2 (A and B) shows that the active anthranilate synthetase in strain trpD80 sediments as fast as the native anthranilate synthetase complex of wild type, indicating that the anthranilate synthetase CoI of strain trpD80 forms a complex with the anthranilate synthetase CoI\textsuperscript{CRM} of this strain. It is also apparent that the altered anthranilate synthetase CoI (CoI\textsuperscript{CRM}) produced by the strain carrying E gene missense mutation trpE3 is probably complexed with anthranilate synthetase CoII (Fig. 2C). In this case, PR-transferase sediments at the same rate as the anthranilate synthetase complex. Uncomplexed anthranilate synthetase CoI and CoII both sediment more slowly than does alkaline phosphatase. Similar activity distributions were noted in experiments employing Sephadex gel filtration. These findings demonstrate that CRM-forming and CRM-less mutants of either the D or E gene can be distinguished by sucrose density-gradient centrifugation or Sephadex gel filtration.

Incomplete anthranilate synthetase complex of a deletion mutant. Genetic and biochemical studies with deletion mutants which lack genes A, B, C, and part of D established that some of these mutants accumulate anthranilic acid and produce an active anthranilate synthetase (11). In subsequent investigations, it developed that the anthranilate synthetase complex produced by such strains was not complete; the activity of the complex could be appreciably increased by the addition of normal anthranilate synthetase CoII (Fig. 3). When an extract of an E gene missense mutant was added, however, stimulation was not observed (Fig. 3). This suggests that the anthranilate synthetase CoI\textsuperscript{CRM} and anthranilate synthetase CoII in this missense mutant are so firmly associated that component II is not available for complex formation with the component I of the deletion mutant. Other studies (C. Yanofsky, unpublished data) have shown that under usual conditions the anthranilate synthetase complex is virtually undissociable.

Comparative properties of the anthranilate synthetase complex and anthranilate synthetase CoI. Since the anthranilate synthetase complex and anthranilate synthetase CoI are both enzymatically active when NH\textsubscript{4}\textsuperscript{+} is employed as substrate, their properties could be compared. (i) The effect of chorismate concentration on initial velocity was examined by using purified preparations of the complex and component I (Fig. 4). It is evident that higher concentrations of chorismate are required to saturate component I. The apparent $K_{\text{m}}$ values for chorismate for the
that anthranilate synthetase CoII increases the affinity of component I for chorismate, rather than itself contributing a binding site for chorismate.

Figure 5 shows the effect of (NH₄)₂SO₄ concentration on initial velocity. The apparent $K_m$ values of the complex and component I for NH₄⁺ are not appreciably different, 3.9 × 10⁻³ M and 2.5 × 10⁻³ M, respectively.

(ii) In the accompanying paper (10), we concluded that anthranilate synthetase CoI has both the catalytic site for the anthranilate synthetase reaction and the feedback-sensitive site. It was of particular interest to compare the feedback sensitivity of the anthranilate synthetase complex and anthranilate synthetase CoI to see whether component II modified the tryptophan sensitivity of component I. Figure 6 (A and B) shows the inhibition of initial velocity as a function of L-tryptophan concentration, with fixed concentrations of chorismate or NH₄⁺ (see also Fig. 9, accompanying paper, reference 10). It is apparent that the feedback sensitivity of the complex is greater than that of anthranilate synthetase CoI and inhibition of the complex approaches 100%. These results differ somewhat from those of Baker and Crawford (1), who found that L-tryptophan could not completely inhibit the activity of the anthranilate synthetase complex.

It is interesting to note that the tryptophan sensitivity of the altered anthranilate synthetase produced by the mutant carrying the deletion trpA-Ddel15 is intermediate between that of the complex and component I.

(iii) The differential effects of heat on the activities of the anthranilate complex and anthranilate synthetase CoI are illustrated in Fig. 7.

![Figure 3](image-url)  
**Fig. 3.** Effect of anthranilate synthetase CoII on the activity of the anthranilate synthetase of a deletion mutant. The procedure employed was essentially identical with that described in Fig. 1, except that 0.32 anthranilate synthetase units from an extract of the strain trpA-D-del15 (1.5 mg of protein), and the indicated amount of anthranilate synthetase CoII (PR-transferease) from extracts of strains trpE972 and trpE3 were added. Component II units from strain trpE972 added (●); component II units from strain trpE3 added (○).

![Figure 4](image-url)  
**Fig. 4.** Effect of chorismate concentration on the relative activity of the anthranilate synthetase complex and of component I. Velocity = units of activity per ml. The ammonium sulfate concentration employed was 0.12 M; chorismate concentrations varied as indicated. Anthranilate synthetase complex, 6 μg (●); anthranilate synthetase CoI, 25 μg (○).

![Figure 5](image-url)  
**Fig. 5.** Double reciprocal plot of initial velocity against ammonium sulfate concentration. The chorismate concentration was 2 × 10⁻³ M; the ammonium sulfate concentration was varied. Anthranilate synthetase complex (●); anthranilate synthetase CoI (○).
When the enzyme preparations are heated at 60 C in a 0.05 M Tris-hydrochloride buffer at pH 7.8, the activity of component I is lost rapidly, whereas the activity of the anthranilate synthetase complex is relatively stable; after 15 min of heating, approximately 80% of the original activity is retained. This finding also demonstrates that the properties of component I are altered when it is present in the complex.

(iv) It was shown previously that Mg²⁺ ions are required for anthranilate synthetase activity (9). The relative effectiveness of various divalent cations in activating the anthranilate synthetase complex and anthranilate synthetase Col alone can be seen by comparing Fig. 8A and 8B. The enzyme preparations employed were dialyzed before use against 0.01 M Tris-hydrochloride buffer at pH 7.8, containing 2 x 10⁻³ M ethylenediaminetetraacetate. Mg²⁺, Co²⁺, and Fe²⁺ are all effective in stimulating activity (Fig. 8). Other divalent cations tested—Mn²⁺, Ca²⁺, Zn²⁺, and Sr²⁺—were without effect.

It is also clear from the data in Fig. 8 that the two enzyme preparations differ markedly in their response to the three cations. Anthranilate synthetase Col is most active in the presence of low concentrations of Co²⁺, whereas the anthranilate synthetase complex is most active in the presence of Mg²⁺. It is interesting to note that Kingdon and Stadtman (13) observed similar differences in the response of the glutamine synthetases of E. coli. Purified glutamine synthetase I was more active in the presence of Mg²⁺ and Co²⁺, whereas synthetase II was more active in the presence of Mn²⁺. These differences have been shown to be due to the presence or absence of covalently bound S'-adenosyl groups (19).

Comparison of the properties of the anthranilate synthetase complex and anthranilate synthetase CoII. Both the anthranilate synthetase complex and anthranilate synthetase CoII catalyze the PR-transferase reaction, the second in the sequence of reactions of tryptophan biosynthesis. Comparative studies were performed with component II and the complex in an effort to determine the effect of component I on the activity and properties of component II. The PR-transferase activity of anthranilate synthetase CoII is relatively constant, except at relatively high concentrations of component I (Fig. 9). The inhibi-
tation seen may not be caused by component I but may be due to an impurity in the partially purified preparation employed.

(i) The effect of PRPP concentration on the PR-transferase activity of the anthranilate synthetase complex and anthranilate synthetase CoI was compared. Both the complex and component II alone give typical substrate saturation curves and do not show cooperative effects. The apparent $K_m$ values for PRPP are approximately the same; $10^{-4}$ M and $2 \times 10^{-4}$ M, respectively, for the complex and component II. The $K_m$ difference may not be significant.

(ii) The PR-transferase activity of the anthranilate synthetase complex is appreciably more heat-stable than the activity of the unassociated anthranilate synthetase CoI (Fig. 10). Both inactivation curves follow first-order kinetics. The same differential effect of heating was observed in comparable studies with these enzymes from S. typhimurium (2).

(iii) It was previously noted that the PR-transferase activity of the anthranilate synthetase complex was inhibited by tryptophan. The transferase activity of anthranilate synthetase CoI is not subject to this inhibition (Fig. 11). Tryptophan inhibition of the transferase activity of the anthranilate synthetase complex was reported for the S. typhimurium and the Aerobacter aerogenes enzymes (2, 5); the inhibition reaches a limiting value and thus may never be complete (Fig. 11). It is clear, therefore, that the inhibitor binding site is distinct from the substrate binding site. The kinetic data presented in Fig. 12 show that the inhibition of PR-transferase activity by L-tryptophan is noncompetitive with respect to PRPP. In other studies, it was shown that L-phenylalanine, L-tyrosine, and D-tryptophan do not inhibit, whereas DL-5-methyltryptophan is slightly inhibitory.

Experiments were also performed to determine whether the tryptophan inhibition of the complex resulted from tryptophan binding to the feedback site on component I. For this purpose, a mutational desensitized anthranilate synthetase CoI was prepared from the strain carrying two mutational changes trpA-D121 and trpEFR 2. The data in Table I demonstrate that component II is inhibited by L-tryptophan when it is complexed with the feedback-resistant component I. This finding suggests either that there is a tryptophan binding site on component II or that tryptophan bound to component I results in inhibition of the transferase reaction and that this function is not affected by the alteration removing feedback sensitivity of component I.

**DISCUSSION**

The results presented in this paper demonstrate that anthranilate synthetase component I, the product of the E gene of the tryptophan operon, is activated not only by normal anthranilate synthetase CoI (PR-transferase) but by a component IIa. and by a presumed fragment of component II produced by a deletion mutant with a deletion terminus in the structural gene for component II, the D gene. In the latter case, activation was not complete; anthranilate synthetase activity was increased when normal component II was added. These observations suggest that, in the activation of component I, component II does not have to provide an active transferase catalytic site.

The finding that component I alone cannot catalyze the reaction chorismate $+\ L$-glutamine $\rightarrow$ anthranilate, whereas this reaction will proceed upon addition of component II, suggests that component II either activates a glutamine binding site on component I or provides this binding site itself. These alternatives remain to be examined. Also, the physiological significance of the fact that $NH_4^+$ can replace glutamine as amino donor in the anthranilate synthetase reaction warrants.
further investigation. It has already been reported that NH$_4^+$ will serve as amino donor in this reaction in vivo (8).

In the comparative studies with the anthranilate synthetase complex and CoI, it was shown that affinity for chorismate, thermostability, divalent cation requirements, and sensitivity to tryptophan inhibition were markedly different. Similarly, in the comparison of the anthranilate synthetase complex and CoII in catalyzing the PR-transferase reaction, differences were found, including the observation that only the complex was sensitive to tryptophan inhibition. This finding was reported previously on the basis of studies with comparable material from *S. typhimurium* (2).

Our investigations provide the additional observation that this inhibition is not related to the feedback sensitivity of component I.

Considering what we now know about the anthranilate synthetase complex and its subunits, it is interesting to point out some obvious differences from the most extensively studied regula-

---

**FIG. 8.** (A) Effect of divalent cations on the activity of the anthranilate synthetase complex. The reaction mixture contained $5 \times 10^{-4}$ M chorismate, $1.5 \times 10^{-2}$ M ammonium sulfate, $5 \times 10^{-3}$ M Tris-hydrochloride buffer (pH 7.8), and the indicated concentrations of MgCl$_2$, FeCl$_2$, and CoCl$_2$ in a final volume of 2 ml. The enzyme preparation used in this experiment was the eluate of a DEAE-Sephadex column. It was dialyzed against 0.01 M Tris-hydrochloride buffer (pH 7.8) containing $2 \times 10^{-3}$ M EDTA for 4 hr before use. (B) Effect of divalent cations on the activity of anthranilate synthetase CoI. The procedure was identical with that described in A, except that dialyzed anthranilate synthetase component 1 was used.
ANTHRANILATE SYNTHETASE COMPARATIVE STUDIES

Fig. 9. Influence of increasing amounts of anthranilate synthetase Col on the PR-transferase activity of a fixed amount of anthranilate synthetase ColI. An eluate from a G-100 Sephadex column of strain trpE5.972 extract was used as the PR-transferase preparation. Partially purified anthranilate synthetase ColI was used (specific activity, 200). PR-transferase activity was assayed.

Fig. 10. Heat inactivation of the PR-transferase activity of the anthranilate synthetase complex and component II alone at 42°C. The incubation mixture contained 0.05 M Tris-hydrochloride buffer (pH 7.8), 2 x 10^{-3} M β-mercaptoethanol, and 15 units of crude anthranilate synthetase complex or 10 units of anthranilate synthetase ColI in a final volume of 1.0 ml. Samples of 0.05 ml each were taken at the indicated time and assayed immediately. PR-transferase was assayed as described in Fig. 9. Anthranilate synthetase complex (●); anthranilate synthetase ColI (○).

Fig. 11. Effect of L-tryptophan on the PR-transferase activity of the anthranilate synthetase complex and component II. The reaction mixture contained 10^{-4} M anthranilate, 10^{-4} M PRPP, 4 x 10^{-3} M magnesium acetate, 2 x 10^{-3} M β-mercaptoethanol, 0.05 M Tris-hydrochloride buffer (pH 7.8), partially purified ColI and complex, and the indicated concentration of L-tryptophan in a final volume of 2 ml. Anthranilate synthetase complex (●); anthranilate synthetase ColI (○).

Fig. 12. Double reciprocal plot of the data of Fig. 11. functional sense because the anthranilate synthetase complex catalyzes two sequential reactions in tryptophan biosynthesis. On the other hand, it is clear that the tryptophan sensitivity of anthranilate synthetase ColI is enhanced when it is complexed with component II. Preliminary investigations on the molecular weight of the E. coli anthranilate synthetase complex, and its subunit composition, give values of 260,000 ± 20,000...
TABLE 1. Effect of L-tryptophan on the PR-transferease activity of anthranilate synthetase CoII and a component IFBR-component II complexa

<table>
<thead>
<tr>
<th>Enzyme component</th>
<th>L-Tryptophan Activity</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>umol/mg</td>
</tr>
<tr>
<td>II</td>
<td>32.3</td>
<td>105</td>
</tr>
<tr>
<td>IFBR + II</td>
<td>32.5</td>
<td>105</td>
</tr>
<tr>
<td>II</td>
<td>5 × 10^{-4}</td>
<td>32.0</td>
</tr>
<tr>
<td>I + II</td>
<td>5 × 10^{-4}</td>
<td>14.2</td>
</tr>
<tr>
<td>IFBR + II</td>
<td>5 × 10^{-4}</td>
<td>16.7</td>
</tr>
</tbody>
</table>

a The anthranilate synthetase CoI and CoII used in this experiment were obtained from crude extracts of strains carrying the mutations trpD-9778 and trpeS972, respectively, and the anthranilate synthetase CoIFBR was obtained from a crude extract of the double mutant carrying trpA-D40121, trpEFBR2. The reaction mixture contained 10^{-2} M anthranilate, 10^{-2} M PRPP, 4 × 10^{-3} M magnesium acetate, 2 × 10^{-2} M 1-mercaptoethanol, 5 × 10^{-3} M Tris-hydrochloride buffer (pH 7.6), and crude extracts of strains trpD9772, trpD9778, or trpA-D40121, and trpEFBR2 (usually 0.05 ml each), and various mixtures thereof.

b With 10^{-4} M chorismate, 10^{-2} M L-glutamate, 4 × 10^{-3} M magnesium acetate, 2 × 10^{-2} M 1-mercaptoethanol, 10^{-2} M potassium phosphate buffer (pH 7.6), and 5 × 10^{-4} M L-tryptophan, the anthranilate synthetase CoIFBR+CoII complex is inhibited approximately 8% in the anthranilate synthetase reaction. The wild-type anthranilate synthetase complex is inhibited completely (Fig. 6B) under these conditions.

and two subunits of each component in the complex (Ito, unpublished data).

Kinetic data indicate that there is no substrate cooperativity either in the complex or subunit-catalyzed reactions, under our conditions. These findings are in agreement with the previously reported conclusions of Baker and Crawford (1). Evidently the anthranilate synthetase complex is not a typical allosteric enzyme (17), although it could be considered an allosteric enzyme of the V type (3).

ACKNOWLEDGMENTS

J. Ito wishes to express his thanks to Dr. Yanofsky and the members of his group at Stanford for their hospitality and encouragement. J. Ito is indebted to Dr. Matsushiro for his support of the continuation of this project. This work was supported by grants from the Public Health Service (GM-09738) and from the National Science Foundation (GB-6790).

LITERATURE CITED


7. Gerhart, J. C., and H. K. Schachman. 1968. Allosteric in-


15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Rand-


17. Monod, J., J. Wyman, and J. P. Changeux. 1965. On the na-


20. Yanofsky, C. 1957. Enzymatic studies with a series of try-