ADVANCES IN ENZYMOLOGY

AND RELATED AREAS OF MOLECULAR BIOLOGY

Founded by E. F. NORD

Edited by ALTON MEISTER

VOLUME 38

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CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK

VOLUME 38

1973
AN INTERSCIENCE® PUBLICATION

JOHN WILEY & SONS
New York • London • Sydney • Toronto

An Interscience® Publication

Library of Congress Catalog Card Number: 41-9213

ISBN 0-471-59173-4

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

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ANTHRANILATE SYNTHETASE

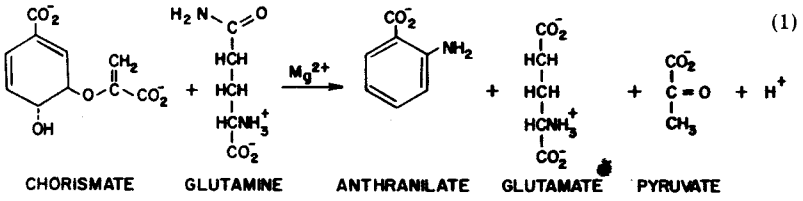
By H. ZALKIN, *Lafayette, Indiana*

CONTENTS

I. Introduction

Anthranilate synthetase catalyzes the first specific reaction for tryptophan synthesis in all microorganisms thus far studied (1) and perhaps also in plants (2). This reaction is shown in equation 1. Similar to other glutamine amidotransferases (3), NH_3 can replace glutamine, in which case the products of the reaction are anthranilate, pyruvate, and H_2O.

Anthranilate synthetase enzymes from all species so far examined are oligomeric proteins containing nonidentical subunits. The subunits are designated anthranilate synthetase Components I and II (4). Both subunits are required for glutamine-dependent



(1)

enzyme activity, but anthranilate synthetase Component I, by itself, catalyzes product formation using NH_3 as amino donor. Recent evidence, to be reviewed, indicates that anthranilate synthetase Component II provides the glutamine binding site.

Anthranilate synthetase enzymes from most species are subject to end product inhibition by tryptophan. Tryptophan binds to anthranilate synthetase Component I.

II. Aggregates of Anthranilate Synthetase

Multifunctional enzymes or enzyme aggregates have been detected in the tryptophan pathway of many organisms. However, in species of *Pseudomonas* (5), *Chromobacterium violaceum* (6), and *Bacillus subtilis** (7) aggregates or multifunctional enzymes are not found. Several patterns of association of anthranilate synthetase have been reported. These patterns are summarized in Table I. In bacteria, two types of anthranilate synthetase have been recognized (9). Type I anthranilate synthetases are oligomeric proteins not associated with other enzymes of the tryptophan biosynthetic pathway. Enzymes of type I have been isolated from *C. violaceum* (6), *B. subtilis* (10,11), species of *Pseudomonas* (12), and *Serratia marcescens* (9). Type II anthranilate synthetases are oligomeric proteins found in association with the second enzyme of the tryptophan biosynthetic pathway anthranilate - 5 - phosphoribosylpyrophosphate phosphoribosyltransferase (PR transferase). Enzymes of type II have been isolated and at least partially characterized from *Escherichia coli* (13,14), *Aerobacter aerogenes* (15,16), and *Salmonella typhimurium* (17,21).

* A contrary conclusion has been reported (8), but the most direct evidence argues against aggregate formation for tryptophan biosynthetic enzymes in *B. subtilis*.

A third type of anthranilate synthetase has been detected in yeast and fungi (22,23). In these organisms anthranilate synthetase is normally isolated in association with indole glycerol 3-phosphate synthetase or with indole glycerol 3-phosphate synthetase and *N*-(5′-phosphoribosyl)anthranilate isomerase activities. It is apparent from this summary that anthranilate synthetase may or may not be associated with other enzymes of the tryptophan pathway and that tryptophan synthetase is the only enzyme of the pathway not aggregated with anthranilate synthetase in any organism.

The summarized data on subunit composition and molecular weights in Table I are discussed in the next section.

III. Subunit Composition of the Various Types of Anthranilate Synthetase

In no case has the subunit composition of anthranilate synthetase from any organism been documented with unequivocal physiochemical evidence. Furthermore, in some instances deductions are made from studies with crude or partially purified enzymes. Nevertheless it appears that a compilation and analysis of presently available data may provide insights into structure-function and evolutionary relationships. For example, it appears that all anthranilate synthetase enzymes are oligomeric proteins containing nonidentical polypeptide chains. These protein chains are designated anthranilate synthetase Components I and II (4). Each component contributes specialized functions to the enzymatic reaction.

A. TYPE I

Anthranilate synthetase enzymes in this class are not aggregated to other proteins of the tryptophan pathway, according to gel filtration or sucrose gradient centrifugation analyses made on crude extracts. In addition other enzymes of the tryptophan pathway are removed upon partial or complete purification of anthranilate synthetase. It is of course possible that subtle physiologically important associations are destroyed upon cell disruption.

TABLE I

Patterns of Aggregation and Subunit Composition of Anthranilate Synthetase in Microorganisms[a]

Type	Organism	Activities aggregated to anthranilate synthetase	Subunit composition[b]	Molecular weight		Reference
				Component I	Component II	
1	*Bacillus subtilis*[c]	None	I_1II_1	80,000[d]	16,000	11
	Chromobacterium violaceum	None	—	—[e]	—	6
	Pseudomonas putida	None	I_1II_1	64,000	18,000	12
	Pseudomonas aeruginosa	None	I_1II_1	64,000	18,000	12
	Pseudomonas acidovorans	None	I_2II_2	71,000	18,000	12
	Pseudomonas testosteroni	None	I_2II_2	71,000	18,000	12
	Serratia marcescens	None	I_2II_2	60,000	21,000	9
	Salmonella typhimurium[f]	None	I_2II_2*	64,000	15,000–19,000	26
2	*Aerobacter aerogenes*	PR transferase	—	—	—	15, 16
	Escherichia coli	PR transferase	I_2II_2	60,000	60,000	13, 14
	Salmonella typhimurium	PR transferase	I_2II_2	64,000	63,000	21
3	*Aspergillus nidulans*	InGP synthetase and PRA isomerase	—	—	—	22
	Neurospora crassa	InGP synthetase and PRA isomerase	$I_2II_2III_4$[g]	40,000	30,000	23, 29
	Saccharomyces cerevisiae	InGP synthetase	—	—	—	22, 27

[a] Modified from Henderson (24).

[b] Anthranilate synthetase Components I and II are abbreviated I and II. Other abbreviations are PR transferase, anthrani-

late-5-phosphoribosylpyrophosphate phosphoribosyltransferase; InGP synthetase, indole glycerol 3-phosphate synthetase; PRA isomerase, *N*-(5′-phosphoribosyl) anthranilate isomerase.

[c] Anthranilate synthetase from *B. alvei* (25) may be analogous based on similarities of molecular weight.

[d] Calculated from the difference between the approximate molecular weight of the oligomeric enzyme and that of anthranilate synthetase Component II. This value and the subunit composition are highly provisional, as noted in the text.

[e] Subunit molecular weight was not estimated. A value of 80,000 to 95,000 was estimated for the intact enzyme.

[f] Native type II anthranilate synthetase that has been digested with trypsin. A fragment of II designated II* is associated with I.

[g] III designates PRA isomerase-InGP synthetase. The number of II subunits was not specified by the original authors (29) but has been tentatively indicated as 2 for this table (see text).

Anthranilate synthetase from crude extracts of *Serratia marcescens* was shown by Hutchinson and Belser (30) to separate from other tryptophan biosynthetic enzymes following sucrose gradient centrifugation. The enzyme was later purified to homogeneity (9,31). A molecular weight of approximately 140,000 was estimated by sucrose gradient centrifugation (9,31) while a value of approximately 150,000 was obtained by gel filtration (31). Gel electrophoresis in urea or sodium dodecyl sulfate revealed the presence of nonidentical polypeptide chains (9). A typical result is shown in Figure 1. Molecular weights of approximately 60,000 and 21,000 were estimated for the polypeptide chains by sodium dodecyl sulfate gel electrophoresis. In accord with the nomenclature introduced by Ito and Yanofsky (4) the large subunit was provisionally designated anthranilate synthetase Component I

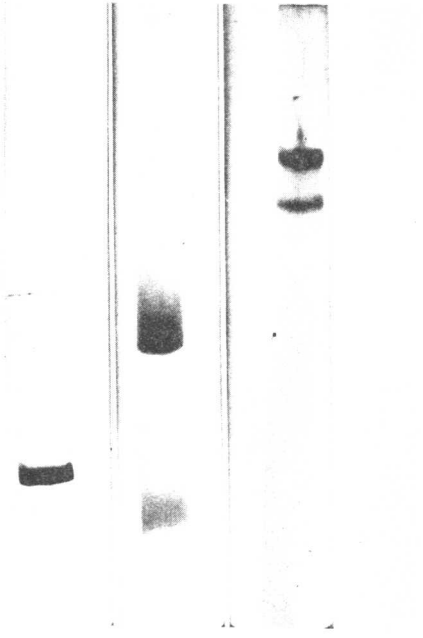


Fig. 1. Gel electrophoresis of anthranilate synthetase from *S. marcescens*. *Left,* homogeneous native enzyme; *middle,* 0.1% sodium dodecyl sulfate plus 0.1% mercaptoethanol; *right,* 8 *M* urea.

and the small subunit anthranilate synthetase Component II. According to this nomenclature unaggregated anthranilate synthetase Component I should contain sites for chorismate and NH_3 and therefore should catalyze anthranilate formation from these substrates. The unaggregated component I should be inactive with glutamine and reactivity with glutamine should be restored upon addition of anthranilate synthetase Component II. These criteria have yet to be fulfilled for the enzyme from *S. marcescens* since separation of the two types of subunits has not been achieved. However other evidence to be described justifies application of this nomenclature.

Evidence supporting a subunit composition of two polypeptide chains of each component (I_2II_2), as shown in Table I, was obtained from intramolecular cross-linking and affinity labeling experiments (9). Most of the eight species expected from random intramolecular crosslinking of a tetramer containing subunits of two sizes using dimethylsuberimidate (32) were detected by sodium dodecyl sulfate gel electrophoresis. Significantly, a protein of molecular weight approximately 40,000 was found following treatment with dimethylsuberimidate suggesting that the oligomeric enzyme might contain two chains of anthranilate synthetase Component II. Further evidence for two chains of anthranilate synthetase Component II was provided by affinity labeling with the glutamine analog 6-diazo-5-oxonorleucine (DON) and with iodoacetamide. Evidence to be presented in Section IV indicates that DON (and by analogy glutamine) bind to a sulfhydryl group of anthranilate synthetase Component II. Approximately 2 moles of DON and iodoacetamide were incorporated per mole of enzyme. Assuming one site per polypeptide chain, this result indicates two chains of anthranilate synthetase Component II per enzyme. Two chains of anthranilate synthetase Component I per enzyme molecule are expected based on a molecular weight of 140,000 to 150,000 for the oligomer. There is reasonable agreement between the sum of the approximate molecular weights of the polypeptide chains assuming a tetrameric composition of I_2II_2 (162,000) and the experimental determination of 140,000 to 150,000 for the oligomer.

Anthranilate synthetase enzymes from species of *Pseudomonas* were studied by Queener and Gunsalus (12,33). An oligomeric

enzyme of molecular weight approximately 65,000 to 73,000 was obtained from *Pseudomonas putida.* Nonidentical polypeptide chains of molecular weight approximately 64,000 and 18,000 corresponding to anthranilate synthetase Components I and II were purified to homogeneity. From this organism the subunits dissociate readily and it was demonstrated that anthranilate synthetase Component I catalyzes the reaction with chorismate and NH_3 as indicated.

$$\text{Chorismate} + NH_3 \xrightarrow{Mg^{2+}} \text{anthranilate} + \text{pyruvate} + H_2O \qquad (2)$$

No activity with glutamine was obtained. Upon addition of anthranilate synthetase Component II reactivity with glutamine was restored. It was concluded that upon interaction of subunits an oligomer of one chain of each component was formed (I_1II_1) as shown in Table I.

A study of anthranilate synthetase enzymes (12) from *Pseudomonas aeruginosa, Pseudomonas acidovorans,* and *Pseudomonas testosteroni* showed that the enzymes could be divided into two groups: *putida-aeruginosa* (p-a class) and *acidovorans-testosteroni* (c-t class). Enzyme from *Pseudomonas stutzeri* resembled the p-a class and *Pseudomonas multivorans* the c-t class. The c-t class anthranilate synthetase Components I and II separated with difficulty, and the aggregate appeared to be larger than the more freely dissociable p-a complexes. Sucrose gradient centrifugation analyses of crude extracts suggested a molecular weight of approximately 155,000 for the oligomeric enzymes from the c-t class. Partially purified anthranilate synthetase Component I from this class was of molecular weight approximately 71,000 while the anthranilate synthetase Component II was similar in size to that from the p-a class, molecular weight approximately 18,000. On the basis of size relationships a composition of I_2II_2 was suggested for the c-t class anthranilate synthetase. Aggregation of isolated components to form oligomer, as measured by appearance of glutamine-dependent anthranilate synthetase activity, was most efficient using enzyme components of the same class yet interclass complementation was obtained. Thus various hybrid enzymes were presumably formed. These relationships are summarized in Table I.

An oligomeric anthranilate synthetase of molecular weight approximately 96,000 containing nonidentical subunits was detected in *Bacillus subtilis* (10.11). Anthranilate synthetase Component II (dubbed "subunit-X") of molecular weight approximately 16,000 was isolated and partially purified but free component I was not obtained. The results were complicated by nonlinearity between enzyme concentration and velocity of glutamine-dependent anthranilate synthetase under conditions of excess component II. Anthranilate synthetase Component II stimulated crude or partially purified glutamine-dependent enzyme activity of the aggregate and restored glutamine-dependent activity to the aggregate from a mutant strain containing inactive component II. These results suggest that *(a)* the aggregate of molecular weight approximately 96,000 may not have been fully saturated with anthranilate synthetase Component II and *(b)* active component II exchanged with the inactive component in the aggregate from the mutant strain. Anthranilate synthetase from *B. subtilis* may be an oligomer of composition I_1II_1 as shown in Table I, but this speculation by the reviewer is tenuous and a more confident description of the enzyme must await further experimentation.

A study of the mutant strain of *B. subtilis,* which contains defective anthranilate synthetase Component II, has provided several interesting additional results (11). *Trp*X which encodes anthranilate synthetase Component II was unlinked by DNA transformation or transduction analyses to the tryptophan gene cluster, yet synthesis of component II was regulated coordinately with the tryptophan synthetase B protein under most conditions (11). Since *trp*X mutants lacking anthranilate synthetase Component II were prototrophic for tryptophan, *in vivo* utilization of NH_3 was suggested. In addition a role was suggested for anthranilate synthetase Component II in the synthesis of 4-aminobenzoate. This is discussed in Section X.

Molecular weight estimations of approximately 80,000 to 95,000 were made for relatively crude preparations of anthranilate synthetase from *Bacillus alvei* (25) and *Chromobacterium violaceum* (6). The enzyme from *C. violaceum* was clearly separated from other enzymes of tryptophan biosynthesis but such information has not been verified for *B. alvei* anthranilate

synthetase. Although no data on subunit composition are yet available, a composition of I_1II_1 could be accommodated by the estimated molecular weights and by analogy to other anthranilate synthetase enzymes described in this section.

Trypsin-treated anthranilate synthetase from *S. typhimurium* is a type I enzyme prepared *in vitro* from the native type II oligomer and is discussed in the next section. As noted in Table I, there is little variation in the approximate molecular weights for type I anthranilate synthetase Components I and II.

B. TYPE II

Anthranilate synthetase enzymes designated type II are aggregated to the second enzyme of the tryptophan pathway, PR transferase. Anthranilate synthetase–PR transferase aggregates have been characterized to varying extents from *E. coli*, *A. aerogenes*, and *S. typhimurium*.

The report by Ito and Yanofsky (4) on "The Nature of the Anthranilic Acid Synthetase Complex of *Escherichia coli*" provided the starting point for all subsequent investigations on the subunit composition of this enzyme. Anthranilate synthetase in extracts of *E. coli* was characterized as an oligomer containing nonidentical subunits. The subunits are the products of the *E. coli trp*E and *trp*D genes. The *trp*E gene product obtained from a *trp*D nonsense mutant was designated anthranilate synthetase Component I. Anthranilate synthetase Component I had a sedimentation coefficient of approximately 4.3 S and lacked glutamine-dependent enzyme activity. It was reported that this protein was "activated" by NH_4^+ ions. It is now recognized that anthranilate synthetase Component I utilizes NH_3 as a substrate together with chorismate for synthesis of anthranilate and pyruvate. The gene product of *trp*D, PR transferase ($s_{20,w}$ approximately 4.4 S) was obtained from a *trp*E nonsense mutant and was required for glutamine-dependent anthranilate synthetase activity in association with anthranilate synthetase Component I. PR transferase was thus designated anthranilate synthetase Component II. Anthranilate synthetase-PR transferase obtained from wild type cells or by mixing extracts containing each of the two components had a sedimentation constant of approximately 7.5 S. These results established that nonidentical

subunits are required for glutamine-dependent anthranilate synthetase activity in *E. coli.*

The molecular weight of essentially homogeneous *E. coli* anthranilate synthetase Component I was reported to be approximately 60,000 to 63,000 (34). The molecular weight of *E. coli* anthranilate synthetase Component II has not been reported. Ito and Yanofsky have stated (13) that preliminary investigations on the molecular weight of the *E. coli* anthranilate synthetase complex, and its subunit composition, give values of 260,000 ± 20,000 and two subunits of each component in the complex.

Anthranilate synthetase PR transferase has been highly purified from *A. aerogenes* (15,16). A sedimentation constant ($s_{20,w}$) of 8.1 S for the aggregate and 4.1 S for anthranilate synthetase Component II was reported (16). Information on the subunit composition of anthranilate synthetase–PR transferase from *A. aerogenes* is not available.

The evidence pointing to a subunit composition of I_2II_2 for anthranilate synthetase–PR transferase from *S. typhimurium* is presently the most complete for any anthranilate synthetase enzyme. The evidence is based on a consideration of the molecular weights of the isolated components and the intact aggregate, stoichiometry of ligand binding, and desitometric analysis following electrophoretic separation and staining of subunits.

On the basis of *in vitro* complementation experiments similar to those performed with extracts of *E. coli* (4), Bauerle and Margolin (17) deduced that anthranilate synthetase from *S. typhimurium* was an oligomer containing nonidentical subunits: anthranilate synthetase Component I catalyzed product formation from chorismate and NH_3 and anthranilate synthetase Component II conferred glutamine reactivity upon component I. Component II by itself also contained PR transferase activity.

Homogeneous anthranilate synthetase Component I was characterized as a single polypeptide chain ($s^\circ_{20,w}$ = 3.8 S) of molecular weight 64,000 (35). This conclusion was based on finding similar molecular weights for the native and denatured component I using gel filtration (36), sedimentation equilibrium centrifugation in the presence and absence of 8 *M* urea or 6 *M* guanidine hydrochloride (35) and sodium dodecyl sulfate gel electrophoresis. Anthranilate synthetase Component II (PR transferase)

was reported to be a polypeptide chain of molecular weight approximately 63,000 but suggestions of aggregation were noted (19). Recent genetic studies also indicate that PR transferase is a single polypeptide chain (37). Electrophoresis of anthranilate synthetase–PR transferase in urea and sodium dodecyl sulfate confirms the presence of nonidentical subunits of similar size. Disc gel electrophoresis in 8 *M* urea reveals two protein bands corresponding to the isolated components (20,21). Sodium dodecyl sulfate gel electrophoresis, on the other hand, yields a single protein band of molecular weight approximately 62,000 (21). It appears unlikely that protein constituents other than anthranilate synthetase Components I and II could be contained in the aggregate.

The relative weight contributions of anthranilate synthetase Components I and II to the aggregate were estimated by integration of densitometer tracings of gels that were stained with Coomassie blue. The relative weight ratio of anthranilate synthetase Component I to Component II was 1.16. Since the subunits have similar molecular weights, equal weight contributions indicate equal numbers of polypeptide chains. A major assumption for these calculations is identical staining of subunits. Although there are elements of uncertainty in this assumption (38), similar analyses have provided well accepted conclusions about the subunit composition of *E. coli* RNA polymerase (39) and *E. coli* aspartate transcarbamylase (40). Supporting evidence for equal numbers of subunits was obtained from measurements of ligand binding. Binding of 1.9 moles of chorismate or of 1.8 moles of tryptophan per mole of enzyme was detected by equilibrium dialysis measurements (21). Chorismate and the feedback inhibitor tryptophan each bind to anthranilate synthetase Component I. Binding of approximately 2 moles of the glutamine analog DON per mole enzyme was shown to be due to covalent attachment to anthranilate synthetase Component II (20). Assuming one site per chain these results indicate two chains each of anthranilate synthetase Components I and II per enzyme molecule.

Molecular weight determinations also are compatible with a composition of I_2II_2 for the *Salmonella* enzyme. The best estimation of molecular weight for the aggregate is 280,000 determined by sedimentation equilibrium centrifugation (21). Other values

of approximately 261,000 to 290,000 were obtained by sucrose gradient centrifugation (18,26) and gel filtration (41). These molecular weight determinations are in reasonable agreement with the value of 254,000 calculated for I_2II_2 ($2 \times 64{,}000 + 2 \times 63{,}000$).

Of interest is a reported molecular weight of 137,000 for anthranilate synthetase from *S. typhimurium* (42). The discrepancy between this value and that of 280,000 was resolved when it was found that during purification a treatment with pancreatic lipase which is often contaminated with proteolytic enzymes caused digestion of the native enzyme (26). The decreased size following digestion of the aggregate with pancreatic lipase or trypsin resulted from digestion of anthranilate synthetase Component II from molecular weight approximately 63,000 to approximately 15,000 to 19,000. Loss of PR transferase activity occurred concomitantly. Anthranilate synthetase Component I was unaltered. It therefore follows that a component II fragment of molecular weight 15,000 to 19,000 can interact with component I and allow glutamine reactivity. It appears that the component II fragment from *S. typhimurium* and the component II chains of similar molecular weight from type I anthranilate synthetase enzymes function as a glutamine binding protein (Section IV). Trypsin-digested anthranilate synthetase from *S. typhimurium* has been included in Table I as a type I enzyme for comparison with those that occur *in vivo*.

Although characterization of the subunit composition has progressed furthest with anthranilate synthetase from *S. typhimurium* the enzymes from *E. coli* and *A. aerogenes* may be similar. *In vitro* complementation experiments (43) indicate facile formation of hybrid anthranilate synthetase aggregates using mixtures of subunits from the three organisms.

C. TYPE III

Anthranilate synthetase enzymes in this category are aggregated to either *N*-(5′-phosphoribosyl)anthranilate isomerase and indole glycerol 3-phosphate synthetase or to just the latter. It appears that such aggregates are found mainly in fungi and yeast although apparently not all fungi have multifunctional aggregates of anthranilate synthetase (22). The basic association appears to