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An Anthranilate Synthase of the Extreme Aminase Type in a Species of Blue-Green Bacteria (Algae)

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Anthranilate synthase of Agmenellum quadruplicatum, *a unicellular species of blue-green bacteria, consists of two nonidentical subunits. A 72,000 dalton protein has aminase activity but is incapable of reaction with glutamine (amidotransferase) unless a second protein (18,000 molecular weight) is present. The small subunit was first detected through its ability to complement a partially purified aminase subunit from* Bacillus subtilis *to produce a hybrid complex capable of amidotransferase function. Conditions for the function of the heterologous complex were less stringent than for the homologous* A. quadruplicatum *complex. A reducing agent such as dithiothreitol stabilizes the* A. quadruplicatum *aminase subunit and is obligatory for amidotransferase function.* L-*Tryptophan feedback inhibits both the aminase and amidotransferase reactions of anthranilate synthase;* K_i *values of* 6×10^{-8} M *for the amidotransferase activity and* 2×10^{-6} M *for the aminase activity were obtained. The* K_m *value calculated for ammonia (2.2 m*M*) was more favorable than the* K_m *value for glutamine (13 m*M*). Likewise, the* V_{max} *of anthranilate synthase was greater with ammonia than with glutamine. Starvation of a tryptophan auxotroph results in a threefold derepression of the aminase subunit, but no corresponding increase in the small 18,000* $\bar{M}$ *subunit occurs. While microbial anthranilate synthase complexes are remarkably similar overall, the relatively good aminase activity of the* A. quadruplicatum *enzyme may be of physiological significance in nature.*

KEY WORDS: anthranilate synthase; blue-green bacteria; aminase; amidotransferase.

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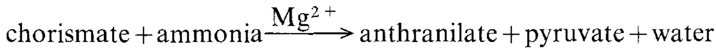
INTRODUCTION

Amidotransferase reactions are essential to the enzymatic formation of amino acids, purines, pyrimidines, amino sugars, and cofactors for ultimate use in the synthesis of protein, nucleic acid, and cell wall macromolecules. Most amidotransferase enzymes may utilize either glutamine or ammonia as the amino-donor reactant. Often an aminase protein enters into association with a glutamine-binding protein to form a glutamine-reactive (amidotransferase) enzyme complex. The significance of ammonia reactivity *in vivo* is unclear. If aminase activity merely represents an evolutionary remnant of a simpler ancestral enzyme, then the ubiquitous conservation of ammonia reactivity among modern amidotransferase proteins is perhaps surprising. Although the high *p*H optimum (*p*H 9–10) observed *in vitro* is regarded as unphysiological, several such aminases have been shown to function adequately *in vivo* in the absence of glutamine reactivity. Thus Gibson *et al.* (1967), Kuhn *et al.* (1972), and Jackson and Yanofsky (1974) all demonstrated that the aminase subunit of anthranilate synthase in *Escherichia coli* can function in tryptophan biosynthesis sufficiently well to sustain normal growth. Kane *et al.* (1972), utilizing a mutant of *Bacillus subtilis* lacking a functional glutamine-binding protein, provided strong evidence that anthranilate synthase and PABA synthase were both capable of function *in vivo* as aminases. In each of the above cases glutamine clearly is the preferred substrate for anthranilate synthase in wild-type cells. However, the possibility of a larger role for aminase reactions in other, less-studied microorganisms may be occasioned by their possibly differing intracellular concentrations of ammonia. Such variability could arise from variant patterns of nitrogen metabolism and/or the diverse ecological niches of microbes.

Few, if any, studies of amidotransferase enzymes have been carried out in species of blue-green bacteria (algae). These autotrophic taxa exist in nutritionally dilute environments and exhibit a generally poor utilization of organic molecules, although the latter point has perhaps been overemphasized in the literature. They frequently are capable of nitrogen fixation, a process which directly generates intracellular ammonia. The often-assumed origin of modern amidotransferases from ancient aminase enzymes would be reinforced by the exclusive presence of aminase proteins in the ancient prokaryote species of blue-green bacteria. This appeared to be the case (Ingram *et al.*, 1972) for anthranilate synthase of *Agmenellum quadruplicatum*, a unicellular, marine species of blue-green bacteria, which initially exhibited only aminase activity when crude extracts were assayed for anthranilate synthase. Although more detailed studies of anthranilate synthase presented in this article resulted in the demonstration of both amidotransferase and aminase reactivities, it nevertheless appears that blue-green bacteria may represent one extreme

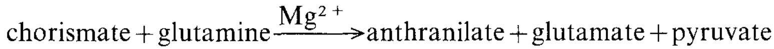
(aminase) in a continuum of microbial enzyme preferences for glutamine or ammonia.

Anthranilate synthase (aminase) catalyzes the following reaction:



$$\text{chorismate} + \text{ammonia} \xrightarrow{Mg^{2+}} \text{anthranilate} + \text{pyruvate} + \text{water}$$

while anthranilate synthase (amidotransferase) catalyzes:



$$\text{chorismate} + \text{glutamine} \xrightarrow{Mg^{2+}} \text{anthranilate} + \text{glutamate} + \text{pyruvate}$$

MATERIALS AND METHODS

Growth Conditions

Cells of *Agmenellum quadruplicatum* strain BG-1 grown as previously described (Ingram *et al.*, 1972) were obtained through the courtesy of Dr. L. O. Ingram (Department of Microbiology, University of Florida, Gainesville, Florida). Tryptophan auxotroph ATR1 was described by Ingram *et al.* (1972).

Extract Preparation

Sedimented cells from the late exponential phase of growth (optical density of 1.4 at 625 nm) were resuspended in 50 mM potassium phosphate buffer, *p*H 7.2, containing 1 mM EDTA and 10 mM dithiothreitol and treated with lysozyme (2.0 mg/ml) for 30 min at 40 C. After sonication (Bronwill) for 30 sec to complete cell disruption, the extract was clarified by centrifugation at 35,000 rpm for 75 min in a Beckman ultracentrifuge. The supernatant was either passed through Sephadex G-25 or dialyzed against buffer at 4 C to remove small molecules.

Enzyme Nomenclature and Abbreviations

The various amidotransferase enzymes considered have the following Enzyme Code Numbers: glutaminase, E.C. 3.5.1.2.; anthranilate synthase, E.C. 2.6.1.*x*; asparagine synthase, E.C. 6.3.5.*x*; carbamylphosphate synthase, E.C. 2.7.2.*x*; CTP synthase, E.C. 6.3.4.2; glutamate synthase, E.C. 1.4.1.*x*; NAD synthase, E.C. 6.3.5.1; FGAR amidotransferase, E.C. 6.3.5.3; PRPP amidotransferase, E.C. 2.4.2.14; XMP amidotransferase, E.C. 6.3.5.2.; D-fructose-6-phosphate amidotransferase, E.C. 2.6.1.16.

The following abbreviations are used: CTP, cytosine triphosphate; PABA, 4-aminobenzoate; FGAR, formylglycinamide ribonucleotide; "H," phosphoribulosylformiminoaminoimidazole carboxamide ribonucleotide; XMP, xanthosine monophosphate; NAD, nicotinamide adenine dinucleotide; CHA, chorismate; gln, glutamine; amn, ammonia.

Enzymology

The aminase and amidotransferase activities of anthranilate synthase were determined fluorometrically (excitation wavelength 313 nm, emission wavelength 393 nm). Routine reaction mixtures for aminase assays contained (in 200 μl) 60 mM tris buffer (*p*H 8.6), 40% (w/v) glycerol, 1mM dithiothreitol, 10 mM $MgSO_4$, 50 mM NH_4Cl, 0.4 mM chorismate, and enzyme as indicated. Amidotransferase activity was assayed in reaction mixtures (200 μl) containing 60 mM tris buffer (*p*H 7.75), 1 mM dithiothreitol, 10 mM $MgSO_4$, 20 mM L-glutamine, 0.4 mM chorismate, and enzyme as indicated. Reactions were started by addition of chorismate to the remaining assay mixture (prewarmed). Specific activity is defined as nmoles of anthranilate formed per milligram of protein at 37 C. Protein concentration was estimated by the method of Lowry *et al.* (1951).

Molecular Weight Determination

Molecular weights were estimated by the method of Andrews (1965) using a 2.5- by 63-cm Sephadex G-200 gel filtration column equilibrated with 50 mM potassium phosphate buffer, *p*H 7.2, containing 1 mM EDTA and 10 mM dithiothreitol. The column was calibrated with the following proteins as molecular weight standards: aldolase (158,000), ovalbumin (45,000), chymotrypsinogen A (25,000), and ribonuclease A (13,700). The void volume was determined with blue dextran.

Biochemicals

Proteins for use as molecullar weight standards were obtained (calibration kit NO DN O1) from Pharmacia Fine Chemicals. Chorismic acid was isolated from the accumulation medium of *Enterobacter aerogenes* 62-1 [reclassified as *Klebsiella pneumoniae* by ATCC (see 1976 ATCC catalogue)] and purified (>95%) as the free acid (Edwards and Jackman, 1965). Glutamine solutions (Sigma) were prepared daily as needed to avoid any possibility of partial degradation to ammonia during storage. Other biochemicals and chemicals of commercial origin were of the highest grade available.

RESULTS

Characterization of Anthranilate Synthase (Aminase) or Anthranilate Synthase (Amidotransferase)

The data in Table I illustrate the requirement of anthranilate synthase for a

Table I. Differential Requirements for Expression and Stability of Amidotransferase and Aminase Activities of Anthranilate Synthase

Enzyme preparation	Addition to reaction mixture	Aminase activity[a]	Amidotransferase activity[b]
		nmoles anthranilate/min/mg	
Original crude extract[c]	None	2.98	2.04
Dialyzed crude extract[d]	None	0.25	—
	Dithiothreitol, 10 mM	0.53	0.53
Stable subunit mixture[e]	None	9.88	2.88
After dialysis[f]	None	1.80	—
	KCl, 0.1 M	1.72	—
	EDTA, 1 mM	2.12	—
	Dithiothreitol, 10 mM	2.24	0.64

[a] Assay reaction mixtures (200 μl) contained 60 mM tris buffer (*p*H 8.6) 40% (w/v) glycerol, 10 mM $MgSO_4$, 50 mM NH_4Cl, 0.4 mM potassium chorismate, enzyme, plus any other additions specified.

[b] Assay reaction mixtures (200 μl) contained 60 mM tris buffer (*p*H7.7), 10mM $MgSO_4$, 20 mM L-glutamine, 0.4 mM potassium chorismate, enzyme, plus any other additions specified.

[c] This extract containing 10 mM dithiothreitol is prepared as described under Materials and Methods and passed through Sephadex G-25. This preparation is stable for at least a week.

[d] A portion of the original extract was dialyzed at 4 C against 2000 vol of 50 mM potassium phosphate buffer (*p*H 7.2), to remove dithiothreitol, and assayed after 24 hr in the presence of the additives indicated.

[e] Subunit mixture contained 16.4 μg of subunit E (aminase) and 22.4 μg of subunit X (glutamine-binding protein), partially purified as illustrated in Fig. 2. The activities of the subunit mixture were stable in 50 mM phosphate buffer (*p*H 7.2) containing 10 mM dithiothreitol.

[f] A portion of the stable subunit mixture was dialyzed for 24 hr at 4 C against 2000 vol of 0.05 M potassium phosphate buffer (*p*H 7.2), and enzyme activities were assayed in the presence of the additives indicated.

sulfhydryl reagent such as dithiothreitol (DTT). EDTA and KCl, present in the assay buffer used and described in a previous report (Kane *et al.*, 1972), did not significantly influence aminase or amidotransferase activity. If DTT is present at the time of extract preparation, both aminase and amidotransferase activities remain stable for at least several months of storage at −80 C. Aminase activity is about 50% greater than amidotransferase activity under the conditions specified. Removal of DTT by dialysis resulted in the complete loss of amidotransferase reactivity and a diminution of aminase activity to less than 10% of the original activity. Readdition of DTT stimulated the residual aminase activity about twofold; but, more dramatically, greater than 25% of the original amidotransferase activity was restored following addition of DTT. The subunit complex therefore exhibits an absolute requirement for DTT in catalysis with glutamine (amidotransferase).

Similar experiments were carried out with partially purified subunit preparations. The results (lines 4–8 of Table I) confirm that DTT is essential for the expression of amidotransferase activity. The decrease in amidotransferase following dialysis, when subsequently assayed in the presence of DTT, is roughly proportional to the irreversible loss of aminase. It appears that DTT stabilizes the aminase, is not essential for storage stability of subunit X, but is essential for formation of an active EX complex capable of amidotransferase function. It is interesting to note that we originally detected the qualitative presence of the glutamine-binding subunit in *A. quadruplicatum* because of its ability to form a hybrid complex with the aminase subunit of *B. subtilis* to yield a functioning amidotransferase [for example, 15.1 μg of partially purified subunit E (aminase) from *B. subtilis* (see Patel *et al.*, 1974) having a specific activity of 7.01 nmoles anthranilate/min/mg with ammonia and no amidotransferase activity with glutamine acquired amidotransferase activity in the presence of glutamine (8.93 nmoles anthranilate/min/mg) following mixture with 0.5mg of crude extract from *A. quadruplicatum*]. In the absence of dithiothreitol, the heterologous complex functions vastly better than the homologous *A. quadruplicatum* complex.

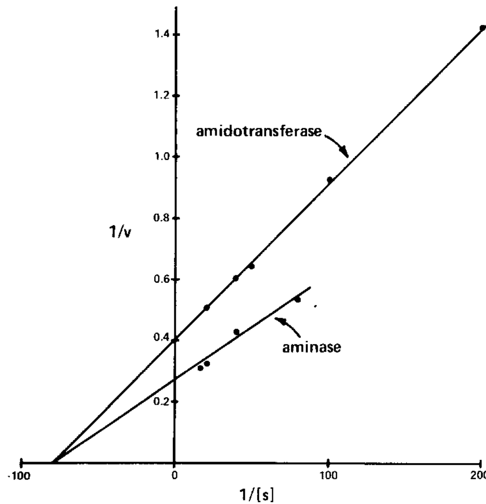


Fig. 1. Double reciprocal plot of anthranilate synthase from *A. quadruplicatum*. The substrate concentration [*s*] denotes M ammonia with anthranilate synthase (aminase) or M glutamine with anthranilate synthase (amidotransferase). A 45-μg amount of crude extract was used to establish the rate indicated at each point. The velocity (*v*) is expressed as nmoles of anthranilate produced/min/mg of protein.

Substrate saturation curves of aminase and amidotransferase activities in a stable crude extract were obtained, and these data are plotted (Fig. 1) in double reciprocal form. Although similar K_m values of 13 mM for glutamine and ammonia were obtained, calculation of the actual concentration of free ammonia present yields a K_m of 2.2 mM for ammonia. The V_{max} for ammonia exceeds that for glutamine.

Nonidentical Subunits

Anthranilate synthase (amidotransferase) was resolved into two nonidentical subunits by gel filtration (Fig. 2). A larger subunit (subunit E) catalyzes the aminase reaction with ammonia. The smaller subunit (subunit X) probably

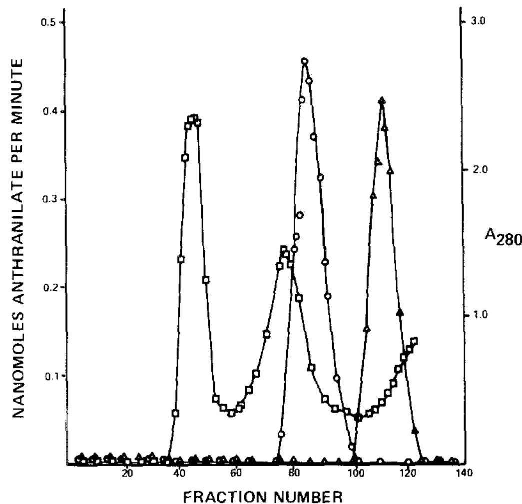


Fig. 2. Fractionation by gel filtration of anthranilate synthase from *A. quadruplicatum*. A 1.0-ml volume of crude extract (38.6 mg protein) was applied to a 2.5- by 63-cm Sephadex G-200 column as described under Materials and Methods. Fractions (2.2 ml) were collected at 4 C. A 50-μl sample of each eluate fraction was assayed for aminase (○) or amidotransferase activities. All amidotransferase assays yielded zero activities, and the experimental points are deleted from the figure so that other symbols on the baseline would not be obscured. The glutamine-binding subunit (△) was located by assay for amidotransferase activity in the presence of 50 μl of the peak fraction (85) corresponding to aminase activity. The relative concentrations of protein in the fractions are plotted on the right ordinate scale as absorbance at 280 nm (□).

binds glutamine. In any event, only the EX complex can function as an amidotransferase in catalysis with glutamine as the nitrogen source for anthranilate synthesis. The molecular weights of subunit E and subunit X are about 72,000 and 18,000 (Fig. 3). Starvation of a tryptophan synthetase A-deficient auxotroph (Ingram *et al.*, 1972) results in a differential derepression (threefold) of subunit E while the concentration of subunit X is unaltered.

The possibility that production of ammonia from glutamine could yield apparent amidotransferase activity as the combined result of independently acting glutaminase and aminase activities was considered. In this case, subunit X would in fact be a glutaminase. This possibility was discounted because (1) direct assays for glutaminase activity in crude extracts or in partially puriffied preparations of subunit X were negative and (2) prior incubation of crude extracts with glutamine did not increase anthranilate synthase (amidotransferase) when the reaction was subsequently initiated with chorismate.

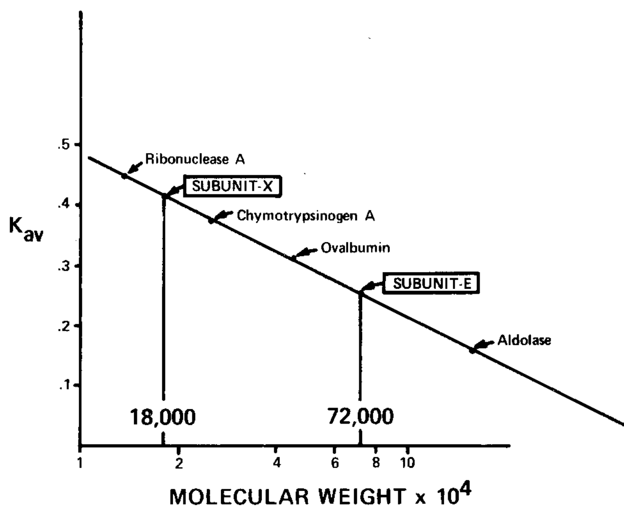


Fig. 3. Estimation of molecular weights for subunit components of anthranilate synthase. A column of Sephadex G-200 was prepared and calibrated as described under Materials and Methods. The assay procedures for identification of the anthranilate synthase subunits are described under Fig. 2. "Subunit E" denotes anthranilate synthase (aminase) and "subunit X" denotes the glutamine-binding subunit, the nomenclature originally used with *B. subtilis* (Kane *et al.*, 1972).The ordinate values are expressed as K_{av}, where $K_{av} = (v_e - v_o)/(v_t - v_o)$, v_e is the elution volume of the protein, v_o is the void volume, and v_t is the total bed volume.

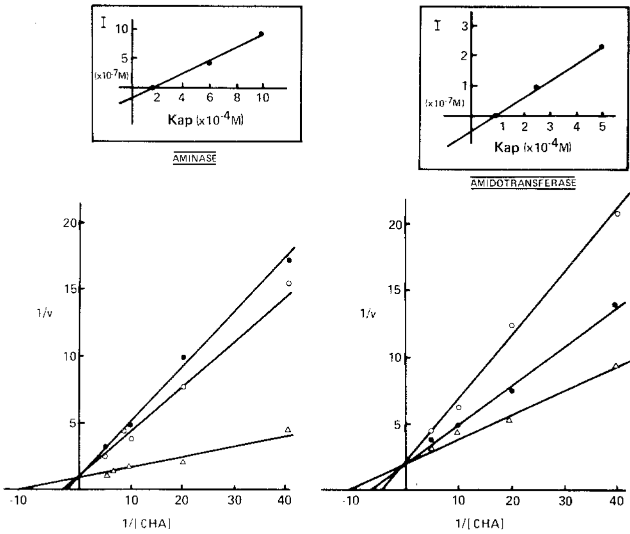


Fig. 4. Effect of L-tryptophan on activity of anthranilate synthase. Each assay mixture contained 190 μg of protein from a crude extract propared as indicated under Materials and Methods. The data shown on the lower left were obtained by assaying aminase activity at the variable concentrations of chorismate (CHA) indicated, using the following concentrations of tryptophan: ▵, no tryptophan; ○, 0.5 μM tryptophan; ●, μM tryptophan. The data on the lower right were obtained by assaying for amidotransferase activity at the variable concentrations of chorismate indicated on the abscissa scale. The concentrations of tryptophan used were ▵, no tryptophan; ●, 0.1 μM tryptophan; ○, 0.25 μM tryptophan. The apparent K_m values (K_{ap}) were determined by plotting reciprocal of velocity (v) against the reciprocal of chorismate concentration (mM) at variable concentrations of inhibitor (I). A plot of I as a function of K_{ap} gives $-K_i$ at the intercept of the ordinate (as shown in the upper left and upper right inserts).

Feedback Inhibition by L-Tryptophan

Both aminase and amidotransferase activities are exceedingly sensitive to inhibition by L-tryptophan (Fig. 4). In each case, inhibition is competitive with respect to chorismate. The K_i values obtained were 0.06 μM and 0.2 μM for amidotransferase and aminase activities, respectively.

DISCUSSION

The ubiquitous capability of amidotransferase enzymes to utilize ammonia raises the question of the significance *in vivo* of aminase function. At one time,

the generalization was tendered (based on comparative enzymological studies of CTP synthase) that ammonia is the source of the amino group in bacterial systems while glutamine is the amino donor for mammalian enzymes (Reichard, 1959). However, CTP synthase (amidotransferase), a function vulnerable to selective inactivation in *Escherichia coli* B, was subsequently demonstrated (Chakraborty and Hurlburt, 1961). Likewise, XMP aminase in *E. coli* (Fukuyama and Moyed, 1964) was later demonstrated to function as XMP amidotransferase as well (Patel *et al.*, 1977). This article shows that anthranilate synthase of *A. quadruplicatum* exhibits more stringent requirements for assay of amidotransferase function than for aminase function. Several apparently exclusively aminase enzyme reactions, e.g., carbamylphosphate synthase of frog liver (Metzenberg *et al.*, 1958), have been reported. There has been a general tendency to discount the *in vivo* role of aminase function because the high *p*H optimum seems unphysiological, especially in cases where the affinity for glutamine is much better than for ammonia. Yet even in these cases such phenomena as the allosteric activation of *E. coli* carbamylphosphate synthase by ammonium ions (Trotta *et al.*, 1974) suggest a role for ammonia under intracellular conditions of low glutamine/high ammonia, a possibility reinforced by the high V_{max} found for ammonia.

Actually, considerable variation is observed in comparing relative reactivities of amidotransferase enzymes with glutamine or ammonia. It is particularly interesting to compare the properties of this large class of enzymes in the single, intensively studied microorganism *E. coli* (Table II). These proteins exhibit a diverse subunit arrangement, many consisting of two nonidentical subunits, but others consisting of a single type of subunit. Subunit molecular weights are highly variable. Likewise, considerable variation exists in enzyme affinities for glutamine or ammonia. It is interesting that the two extreme cases where glutamine reactivity is dominant (glutamate synthase and D-fructose-6-phosphate amidotransferase) are enzymes involving amide transfer to a keto group. Likewise, the other extreme where ammonia reactivity is dominant (asparagine synthase and NAD synthase) is represented by enzymes which both involve amide transfer to a carboxyl group. In many cases the affinity for glutamine is substantially higher than for ammonia, but the V_{max} for ammonia exceeds the V_{max} for glutamine. The ratios of glutamine/ammonia (gln-amn) reported in the literature largely reflect the V_{max} parameter since routine assay procedures involve substrate concentrations that approach saturation.

Anthranilate synthase is perhaps the most widely studied amidotransferase enzyme, and the general similarity of microbial anthranilate synthases is apparent from the summary presented in Table III. This contrasts strikingly with the variability of different amidotransferases present in a single

Table II. Enzymological Properties of Amidotransferase enzymes of *E. coli* [a]

Enzyme	Aminase subunit [b]		Gln-binding subunit [c]	Substrate affinities [d]	V_{max}	Ratio of gln/amn activities	References
Anthranilate synthase	64,000		23,000 [g]	gln > amn	gln = amn [e]	1.9	Edwards *et al.* (1964), Ito and Yanofsky (1966), Li *et al.* (1974), Ito *et al.* (1969), Ito and Yanofsky (1969)
Asparagine synthase		82,000		amn > > gln	amn > > gln	< 0.01	Cedar and Schwartz (1969)
Carbamyl-P-synthase	145,000		48,000	gln > > amn [e]		1.0	Abd-el-al and Ingraham (1969), Trotta *et al.* (1971), Mathews and Anderson (1972)
CTP synthase		50,000		gln > amn		2.5	Chakraborty and Hulburt (1961), Levitzki (1973)
Glutamate synthase	53,000		135,000	gln > > amn	gln > > amn	> 100	Miller and Stadtman (1972), Mantsala and Zalkin (1976)
PABA synthase	48,000		9,000				Huang and Gibson (1970)
FGAR amidotransferase [e]		135,000		gln > > amn			Buchanan (1973)
"H" amidotransferase	41,000		44,000	gln > > amn [f]	gln > > amn [f]		Ames (1973)
PRPP amidotransferase						3.7	LeGal *et al.* (1967)
XMP amidotransferase		126,000		gln = amn	gln = amn	1.6	Patel *et al.* (1977)
NAD synthase				amn > gln		0.6	Spencer and Preiss (1967)
D-Fructose-6-phosphate amidotransferase		100,000		gln > > amn	gln > > amn	> 100	Kornfeld (1967)

[a] See Materials and Methods for abbreviations.
[b] Molecular weight of ammonia (amn)-reactive subunit.
[c] Molecular weight of glutamine (gln)-binding subunit.
[d] K_m or V_{max} values within a factor of 2 denoted =; values differing by a factor of 2–10 demoted >; values differing by more than a factor of 10 denoted > >.
[e] Data from *S. typhimurium*.
[f] See Table I footnotes.
[g] The amino-terminal fragment from phosphoribosyltransferase obtained by controlled proteolysis (Li *et al.*, 1974).

Table III. Amidotransferase and Aminase Characteristics of Microbial Anthranilate Synthases

Organism	Aminase subunit ($\bar{M}$)	Gln-binding subunit ($\bar{M}$)	Substrate affinities[a]	V_{max}[a]	Ratio of gln/amn activities	References
Euglena gracilis	80,000[d]		gln > amn	gln > amn	3.4	Hankins and Mills (1976)
Salmonella typhimurium	64,000	23,000[b]	gln > amn	gln = amn	1.1	Li *et al.* (1974), Tamir and Srinivasan (1969), Henderson and Zalkin (1971)
Escherichia coli	64,000	23,000	gln > amn		1.9	Edwards *et al.* (1964), Ito and Yanofsky (1966) Li *et al.* (1974), Ito *et al.* (1969), Ito and Yanofsky (1969)
Aerobacter aerogenes	60,000	23,000	gln > amn		1.2	Edwards *et al.* (1964), Egan and Gibson (1966)
Serratia marcescens	62,000	21,000	gln > amn		1.3	Robb *et al.* (1971) Robb and Belser (1972)
Pseudomonas putida	63,000	18,000	gln > amn	amn = gln	2.2	Queener *et al.* (1970, 1973)
Bacillus subtilis	67,000	20,000	gln = amn	gln = amn	2.1	Kane *et al.* (1972, 1973), Kane and Jensen (1970), Patel *et al.* (1974), Kane (1975)
Bacillus macerans	62,000	24,000			2.3	Patel *et al.* (1974)
Bacillus licheniformis	> 100,000	24,000			1.9	Patel *et al.* (1974)
Bacillus pumilus	80,000	23,000			1.6	Patel *et al.* (1974)
Bacillus coagulans	80,000	18,000			1.4	Patel *et al.* (1974)
Bacillus alvei	58,000	15,000			0.9	Patel *et al.* (1974)
Agmenellum quadruplicatum	72,000	18,000	amn > gln	amn > gln	0.7	This article
Acinetobacter calcoaceticus	70,000	14,000			0.5	Sawula and Crawford (1973)
Clostridium butyricum[e]	127,000	15,000	gln = amn	gln = amn	0.5	Baskerville and Twarog (1974)

[a] Denoted = if K_m or V_{max} values for glutamine (gln) or ammonia (amn) are within a factor of 2 and > for factors of 2–10. K_m values for ammonia corrected through the use of the Henderson-Hasselbalch equation with *B. subtilis* and *A. quadruplicatum*.
[b] The amino-terminal fragment of phosphoriboyltransferase obtained by controlled proteolysis (Li *et al.*, 1974).
[c] Data from *P. aeruginosa*.
[d] Only one type of subunit exists.
[e] Renamed *Clostridium beijerinckii* (ATCC 6014).

organism (Table II). Except for *Euglena gracilis*, all described anthranilate synthases consist of nonidentical subunits: an aminase (usually 60,000–80,000 $\bar{M}$) and a glutamine-binding subunit (usually about 20,000 $\bar{M}$). The enzyme of *E. gracilis* exemplifies the extreme where glutamine reactivity is dominant. Generally, the K_m is lowest for glutamine and the V_{max} is highest for ammonia.

If it is assumed that ammonia (rather than ammonium ions) is the true substrate entity, then many of the K_m values in the literature could be an order of magnitude too large. A calculation of the fraction of ammonia present at any given *p*H by application of the Henderson-Hasselbalch equation and a pK_a value of 9.3 would provide a more accurate K_m value. Ratios of glutamine/ammonia activities usually range between 1 and 2. *A. quadruplicatum, Acinetobacter calcoaceticus,* and *Clostridium butyricum* all exhibit ratios (gln/amn) of less than unity. With *A. quadruplicatum* the K_m values for ammonia and glutamine are equal while the V_{max} for ammonia exceeds that of glutamine.

Since kinetic parameters of anthranilate synthase from *A. quadruplicatum* are favorable for reaction with ammonia as substrate, aminase function may be more significant in *A. quadruplicatum* than in other microorganisms. It would be interesting to determine whether other amidotransferase enzymes of organisms such *A. quadruplicatum* or *C. butyricum* would parallel anthranilate synthase in possessing enhanced aminase activity, in comparison with data profiles obtained with an organism such as *E. coli* (e.g., Table II).

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