The association of tryptophan synthetase subunits from *Escherichia coli* and *Salmonella typhimurium* in homologous and heterologous combinations

By RALPH FRANCIS DiCAMELLI* AND ELIAS BALBINDER†

Department of Biology, Syracuse University, Syracuse, N.Y. 13210, U.S.A.
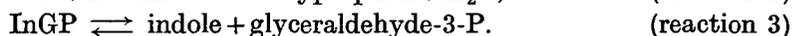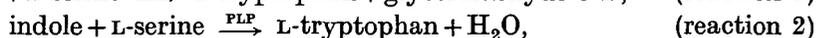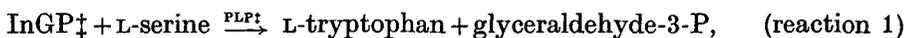
SUMMARY

The association of α and β_2 subunits of tryptophan synthetase from *Escherichia coli* and *Salmonella typhimurium* in homologous and heterologous combinations was studied by sucrose density gradient centrifugation. Under conditions allowing for optimal association of subunits derived from the same source, subunit association in the mixture *E. coli* α–*S. typhimurium* β_2 was weaker than normal while in the reciprocal combination of *S. typhimurium* α–*E. coli* β_2 it was tighter than normal.

These observations suggest that a certain degree of binding between the α and β_2 subunits of tryptophan synthetase could have had a selective advantage during the evolutionary divergence of the species of Enterobacteriaceae, so that a mutation leading to the substitution of an amino acid involved in α–β_2 association in one of the subunits could have been compensated by a mutation in the complementary one.

1. INTRODUCTION

The enzyme tryptophan synthetase [L-serine hydrolase (adding indole) EC 4.2.1.20] has been extensively employed in studies of evolutionary relationships among bacteria and fungi (for recent reviews, see Crawford, 1975*a*, *b*). Biochemical and genetic studies of this enzyme have included comparisons of the primary structures of the α components of bacterial tryptophan synthetases (Guest *et al.* 1967; Li & Yanofsky, 1972, 1973*a*, *b*), and divergences in the nucleotide sequence of some of the corresponding structural genes (Li, Denney & Yanofsky, 1973).

The general properties of tryptophan synthetase have been reviewed by Yanofsky & Crawford (1972). In the Enterobacteriaceae, this enzyme is composed of two non-identical protein subunits, α and β and can catalyse the following three reactions:

$$\text{InGP}\ddagger + \text{L-serine} \xrightarrow{\text{PLP}\ddagger} \text{L-tryptophan} + \text{glyceraldehyde-3-P}, \qquad \text{(reaction 1)}$$
$$\text{indole} + \text{L-serine} \xrightarrow{\text{PLP}} \text{L-tryptophan} + \text{H}_2\text{O}, \qquad \text{(reaction 2)}$$
$$\text{InGP} \rightleftharpoons \text{indole} + \text{glyceraldehyde-3-P}. \qquad \text{(reaction 3)}$$

* Present address: Rush – St Luke Presbyterian Medical Center, 1753 West Congress Pkwy., Chicago, Ill. 60612.

† Present address: American Medical Center at Denver, 6401 West Colfax, Spivak, Colorado 80214, U.S.A. (to whom reprint requests should be addressed).

‡ Abbreviations: PLP, pyridoxal phosphate; InGP, indole-3-glycerol phosphate.

Reaction 1 is the physiologically significant one and it can only be carried out by the fully associated $\alpha_2\beta_2$ enzyme complex. The α subunit is a single polypeptide chain of 268 amino acids with a molecular weight of about 29500 and a sedimentation coefficient of 2·7 S. The β subunit exists as a dimer of two identical polypeptide chains (β_2), which has a molecular weight of about 99000 and a sedimentation coefficient of 5·1 S. Each subunit of the tryptophan synthetase complex has trace catalytic activity in one of the reactions. The α subunit has the ability to catalyse Reaction 3, but only about 1 % as efficiently as the $\alpha_2\beta_2$ complex. The β_2 subunit can catalyse Reaction 2, but only at about 3 % of the activity of the complex. Each individual subunit is inactive by itself in the overall reaction (reaction 1). The fully complexed enzyme $(\alpha_2\beta_2)$ catalyses all three reactions at their maximal rate, has a molecular weight of approximately 159000 and a sedimentation coefficient of 6·4 S. The presence of the substrate L-serine and the coenzyme PLP are required for the formation of the complex (Creighton & Yanofsky, 1966).

Tryptophan synthetase complexes formed between α and β_2 subunits from different enteric bacterial species are quite active (Balbinder, 1964) though differences in affinity between heterologous α and β_2 subunits have been reported (Murphy & Mills, 1969; Rocha, Crawford & Mills, 1972). Sucrose density gradient centrifugation studies of the association of the α and β_2 subunits of *Escherichia coli* (Creighton & Yanofsky, 1966) and its close relative *Salmonella typhimurium* (DiCamelli, Balbinder & Lebowitz, 1973) have shown that the $\alpha_2\beta_2$ complexes from these two enteric bacteria are nearly identical in their sedimentation and dissociation behaviour. Both are subject to pressure effects, i.e. they tend to dissociate when centrifuged at 50000 rev/min rather than at 39000 rev/min, and this tendency can be reversed by increasing the rotor temperature from 5 to 20 °C. This observation indicates that one major force holding the subunits together is hydrophobic bonding (DiCamelli *et al.* 1973). In this report we present the results of sucrose density gradient centrifugation studies of $\alpha_2\beta_2$ complexes obtained in heterologous mixtures of α and β_2 subunits from *E. coli* and *S. typhimurium*. We will show that these 'hybrid' complexes are different from each other as well as from the homologous complexes in their dissociation and sedimentation behaviour.

2. MATERIALS AND METHODS

The *E. coli* and *S. typhimurium* strains used as sources of α and β_2 subunits, as well as the procedures employed for growing bacterial cultures and obtaining partially purified preparations of each subunit have been described (DiCamelli *et al.* 1973). The methods employed in the sucrose density gradient experiments are also described in the same publication. In all cases 5–20 % sucrose gradients prepared in 0·05 M Tris-HCl buffer were used. Except where indicated, these gradients contained 0·02 mM pyridoxal phosphate and 0·15 mM-L-serine, both required for optimal association of α and β_2 subunits (Creighton & Yanofsky, 1966). Fractions were collected and assayed for α and β_2 subunit activity in the indole → tryptophan reaction as described by DiCamelli *et al.* (1973).

The procedures used to assay the enzymic activities of the α and β_2 subunits have been previously described (Smith & Yanofsky, 1962, 1963). All enzyme assays were carried out in a total volume of 1·0 ml at 37 °C for 20 min. In all assays the enzymic activity of one subunit was determined in the presence of at least a threefold excess of the other subunit. For the conversion of indole to tryptophan (reaction 2) the assay mixture was: 0·4 mM indole, 0·04 mM pyridoxal phosphate, 80 mM DL-serine, 100 mM Tris-HCl buffer, pH 7·8, plus 0·03 ml of a saturated solution of NaCl. The disappearance of indole was determined colorimetrically from its reactivity with Ehrlich's reagent after extraction with xylene. The enzymic conversion of indole glycerol phosphate (InGP) to indole (reaction 3, forward) was assayed for the amount of product (indole) formed in 20 minutes at 37 °C. The reaction mixture contained: 0·3 mM InGP, 100 mM Tris-HCl buffer, pH 7·8, and 5 mM hydroxylamine (NH_2OH). The substitution of 100 mM phosphate buffer, pH 7·0, for the Tris-HCl buffer enhanced the usually low activity found in this assay. The addition of hydroxylamine is necessary to inhibit the coupling of L-serine and indole in the crude extracts, thus preventing the conversion of indole to tryptophan (Yanofsky & Rachmeler, 1958). Reaction 1 (InGP $\rightarrow$ tryptophan) was assayed by the disappearance of substrate (InGP). The assay mixture was identical to that used for the indole to tryptophan reaction with the exception that InGP (0·4 mM) was substituted for indole. The amount of unconverted InGP was determined by periodate oxidation to indole-3-aldehyde, which was extracted with ethyl acetate and measured spectrophotometrically at 290 nm.

3. RESULTS

Fig. 1 shows the results of sucrose density gradient centrifugation experiments performed with mixtures of *S. typhimurium* α and β_2 subunits. In the absence of PLP and L-serine two independent peaks were always observed: one for the α subunit ($S_{20,w} = 2\cdot7$ S) and one for β_2 ($S_{20,w} = 5\cdot1$ S) (Fig. 1A). When the mixtures contained an excess of α and were centrifuged at the same speed (39000 rev/min) and rotor temperature (5 °C) in 5–20 % sucrose gradients supplemented with 0·02 mM PLP and 0·15 mM L-serine, a more rapidly sedimenting peak ($S_{20,w} = 6\cdot4$ S) containing approximately equal amounts of α and β_2 activity was observed in addition to the 2·7 S peak corresponding to the excess α subunit (Fig. 1B). The 6·4 S peak has been shown to correspond to the $\alpha_2\beta_2$ complex (Creighton & Yanofsky, 1966; DiCamelli *et al.* 1973). When the same mixture was centrifuged at a speed of 50000 rev/min, complex formation was restricted and only intermediate association was observed (Fig. 1C). Normal association at the same centrifugal speed of 50000 rev/min was restored, however, when the rotor temperature was kept at 20 °C instead of 5 °C during the experiment (Fig. 1D). The pressure increase resulting from the higher centrifugal speed inhibits the association between the subunits, but this association is stabilized by an increase in temperature. Since it is known that hydrophobic bonding will stabilize native proteins better at higher than lower temperatures, these observations indicate that
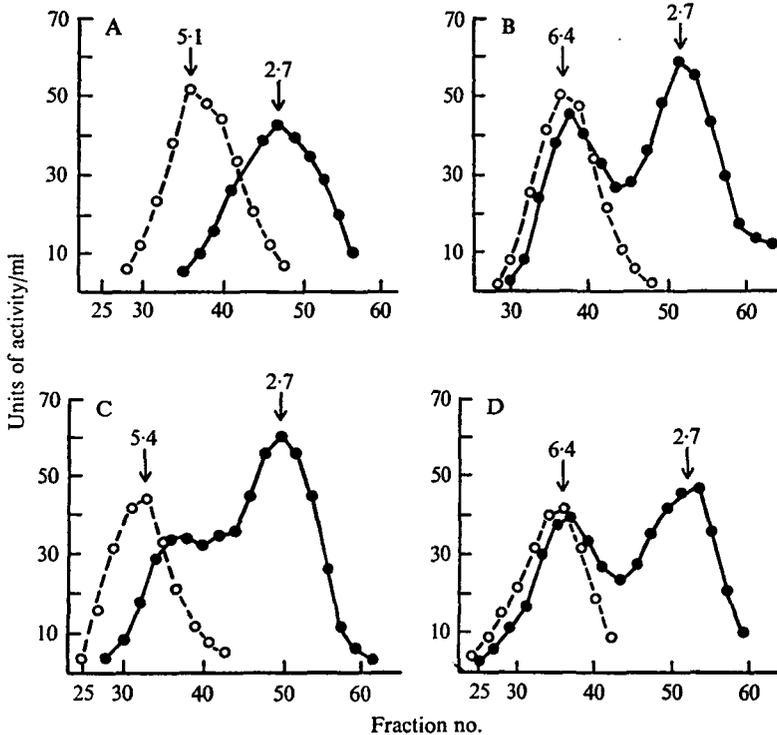
Fig. 1. Sedimentation and association patterns of tryptophan synthetase subunits from homologous mixtures of *S. typhimurium* α and β_2. The α subunit is in excess in B, C and D. (A) 39000 rev/min at 5 °C; PLP and L-serine absent. (B) 39000 rev/min at 5 °C; PLP and L-serine present. (C) 50000 rev/min at 5 °C; PLP and L-serine present. (D) 50000 rev/min at 20 °C; PLP and L-serine present. ●, α subunit activity; ○, β_2 subunit activity.
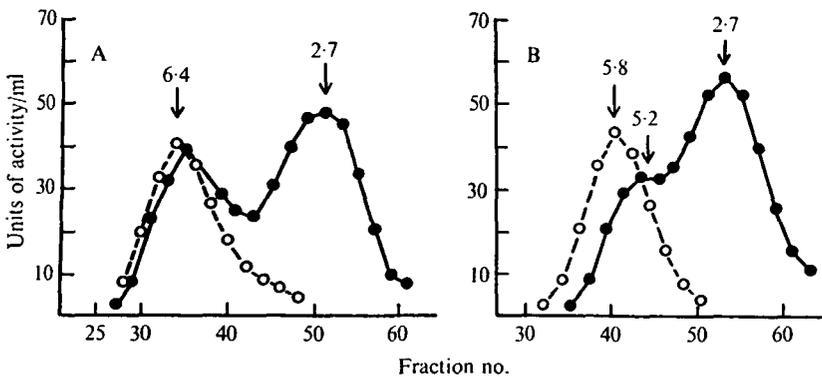

Fig. 2. Sedimentation patterns of heterologous mixtures of tryptophan synthetase subunits from *S. typhimurium* and *E. coli* centrifuged at 39000 rev/min and 5 °C. (A) Excess *S. typhimurium* α subunit plus *E. coli* β_2 subunit. (B) Excess *E. coli* α subunit plus *S. typhimurium* β_2 subunit. ●, α subunit activity; ○, β_2 subunit activity.

hydrophobic bonds are playing an important role in holding native $\alpha_2\beta_2$ complexes together (DiCamelli *et al.* 1973). The same experiments were carried out with homologous mixtures of *E. coli* α and β_2 subunits, and the centrifugation patterns obtained were identical to those shown in Fig. 1 with the exception that the β_2 subunit from *E. coli* is partially inactivated at 50000 rev/min and 5 °C (DiCamelli *et al.* 1973).
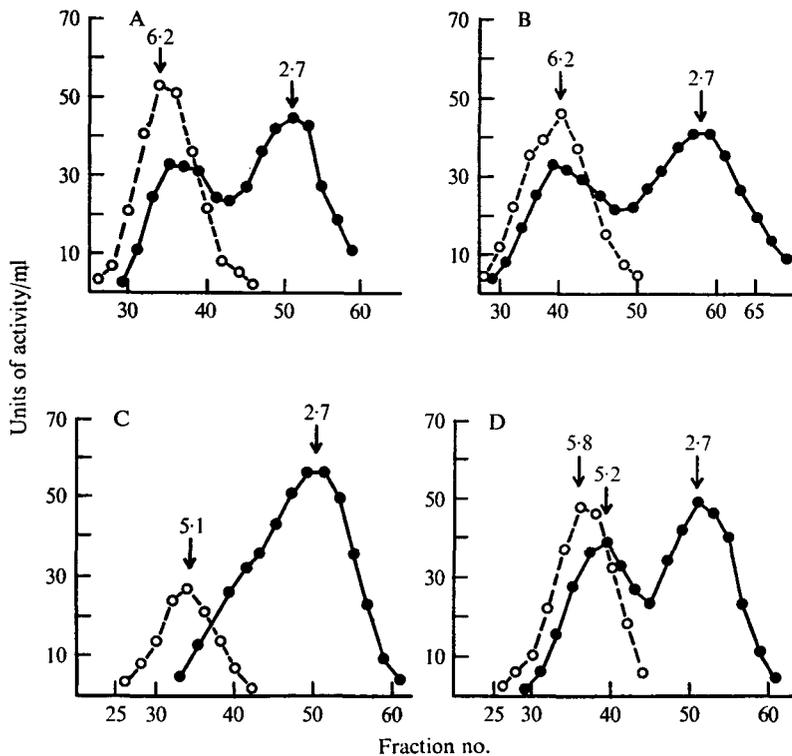

Fig. 3. Effect of pressure and temperature on the sedimentation patterns of mixtures consisting of excess *E. coli* α plus *S. typhimurium* β_2. (A) 39000 rev/min, 12 °C; (B) 39000 rev/min, 20 °C; (C) 50000 rev/min, 5 °C; (D) 50000 rev/min, 20 °C. ●, α subunit activity; ○, β_2 subunit activity.

When *E. coli* β_2 was sedimented with excess *S. typhimurium* α at 39000 rev/min and 5 °C, the resulting pattern was identical to that observed with both homologous combinations (Fig. 2A). However, when the reciprocal combination of *S. typhimurium* β_2 and excess *E. coli* α was sedimented under the same conditions, only intermediate association was observed (Fig. 2B). The $S_{20, w}$ of 5·8 S for β_2 activity is very close to that observed in studies where *E. coli* α was centrifuged in the presence of excess *E. coli* β_2, and shown to correspond to an $\alpha\beta_2$ complex (Goldberg *et al.* 1966; Creighton & Yanofsky, 1966). We did not determine whether the 5·8 S peak we observed corresponded to a hybrid *E. coli* α–*S. typhimurium* β_2 complex or whether the decrease in sedimentation coefficient was simply the result of a general lowered affinity of these subunits for each other. In any case, we never

observed the formation of a normal $\alpha_2\beta_2$ complex when this particular combination of subunits was sedimented under conditions which allow for optimal complex formation in homologous subunit mixtures. Raising the temperature to 12 °C (Fig. 3A) or 20 °C (Fig. 3B) improved the association of the heterologous α and β_2 subunits but optimal complex formation, as evidenced by the appearance of a peak at 6·4 S with equal amounts of α and β_2 activities (as in Fig. 1B) was never observed. There is probably never complete association as evidenced by the lower sedimentation coefficient (6·2 S) and the only partial overlapping of the α and β_2 subunit activities in the region of association. These effects of temperature point again to the contribution of hydrophobic bonding in subunit association, and indicate also that at least some of the non-polar amino acid residues participating in hydrophobic bonding have been conserved during the evolutionary divergence
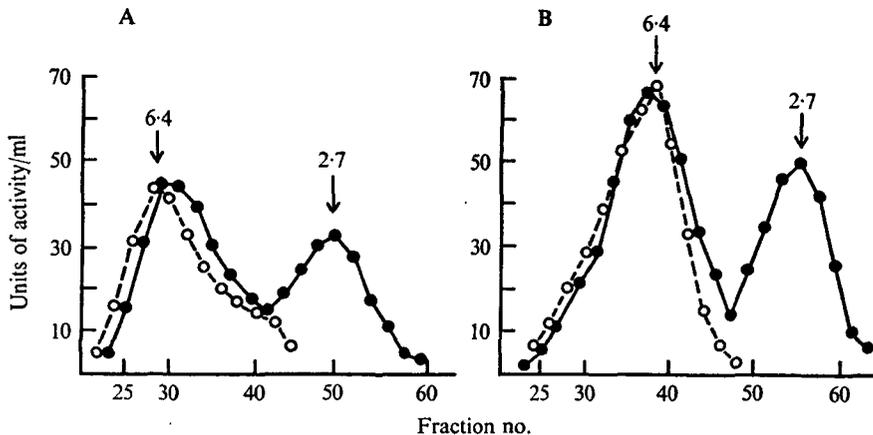

Fig. 4. Effects of pressure and temperature on the sedimentation patterns of the mixture consisting of excess *S. typhimurium* α plus *E. coli* β_2 centrifuged at 50 000 rev/min. (A) At 5 °C, (B) at 20 °C. ●, α subunit activity; ○, β_2 subunit activity.

which led to the two species of enteric bacteria we are concerned with in this report. When this same heterologous mixture (*S. typhimurium* $\beta_2 + E. coli$ α) was subjected to higher pressures at 50 000 rev/min at a temperature of 5 °C each subunit sedimented independently, giving the sedimentation coefficients of 2·7 S for the α subunit and 5·1 S for the β_2 subunit, respectively (Fig. 3C). Thus, the increase in pressure applied to this mixture was sufficient to completely inhibit association. When the temperature was increased to 20 °C keeping the same rotor speed of 50 000 rev/min there was a return to the same degree of intermediate association between α and β_2 seen before at 39 000 rev/min and 5 °C (compare Fig. 3D to Fig. 2B).

The reciprocal heterologous mixture (*S. typhimurium* $\alpha + E. coli$ β_2) seemed to associate normally when centrifuged at 39 000 rev/min and 5 °C (Fig. 2A). Interestingly and surprisingly, when this heterologous mixture was subjected to the higher pressures generated at 50 000 rev/min and centrifuged at low temperature (5 °C) *no* dissociation was observed (Fig. 4A). As was previously shown, the pressure

generated under these conditions was sufficiently strong to partially dissociate homologous mixtures (Fig. 1C) and to completely dissociate the reciprocal heterologous mixture (Fig. 3C). Thus, for this particular combination it seems that there is very strong binding of the heterologous subunits. When the temperature was raised to 20 °C the strong binding was maintained; whether it was enhanced could not be measured with our techniques (Fig. 4B).
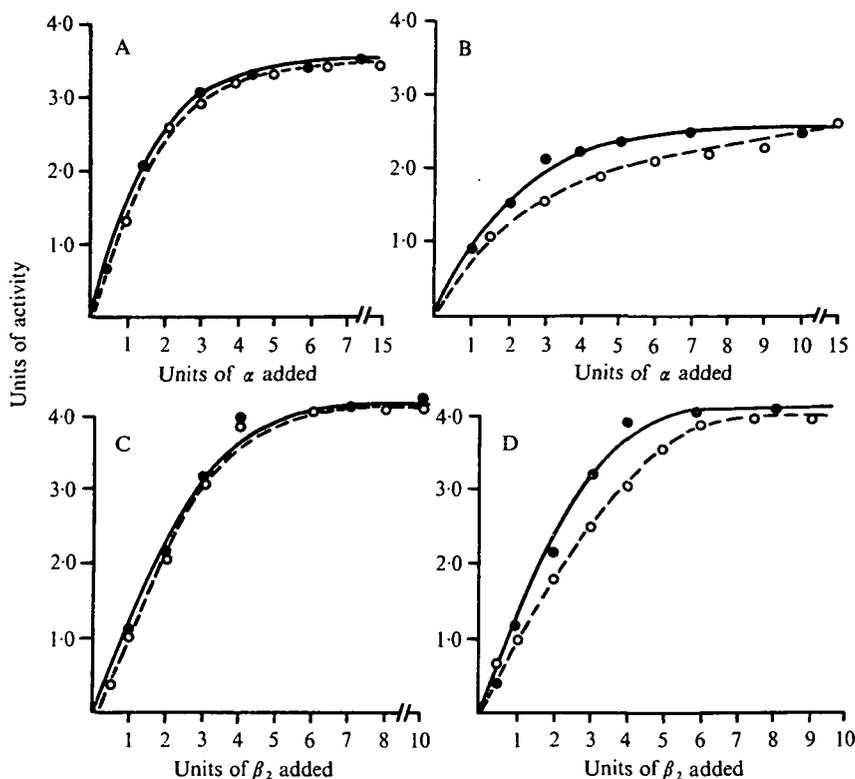

Fig. 5. Saturation curves for α and β_2 subunits from *E. coli* and *S. typhimurium* with homologous and heterologous complementing subunits. Activity measured in reaction 2 (indole → tryptophan). (A) Saturation of 3·5 units of *E. coli* β_2 with increasing amounts of *E. coli* (○) and *S. typhimurium* (●) α subunits. (B) Saturation of 2·6 units of *S. typhimurium* β_2 with increasing amounts of *E. coli* (○) and *S. typhimurium* (●) α subunits. (C) Saturation of 4·0 units of *S. typhimurium* α with increasing amounts of *E. coli* (●) and *S. typhimurium* (○) β_2 subunits. (D) Saturation of 4·0 units of *E. coli* α with increasing amounts of *E. coli* (●) and *S. typhimurium* (○) β_2 subunits.

The differences in the affinity of α and β_2 subunits in the two heterologous combinations that we found by sucrose gradient centrifugation were also observed in saturation experiments (Fig. 5). To a small fixed amount of one subunit were added increasing amounts of the complementing one until saturation was achieved. As Fig. 5 shows, of all possible combinations of subunits only the two between *E. coli* α and *S. typhimurium* β_2 were slow in reaching saturation.

To see whether the differences in association between subunits which we observed in centrifugation experiments would be expressed in an alteration of the catalytic properties of the heterologous $\alpha_2\beta_2$ complexes, we tested the abilities of the two α and the two β_2 subunits to be activated by a homologous or heterologous complementing subunit in all three reactions normally catalysed by the $\alpha_2\beta_2$ complex. The results of these assays are given in Table 1. There was no direct correlation between the strength of subunit association and catalytic activity. However, in all these assays each subunit was assayed in the presence of saturating amounts of the complementing one. These may not be the ideal conditions to detect subtle differences in catalytic properties between homologous and heterologous complexes. Some of our saturation experiments (Figs. 5B, D) suggest that perhaps such differences, if they exist, may be easier to detect if any given subunit is assayed with equivalent, rather than saturating, amounts of the complementing homologous and heterologous subunits. The data presented in Table 1 also show that the *S. typhimurium* α subunit is relatively less efficient in carrying out reaction 3 (InGP → indole) than its *E. coli* counterpart.

Table 1. *Relative rates* of reactions catalysed by homologous and heterologous mixtures of* α *and* β_2 *subunits from* E. coli *and* S. typhimurium

Preparation	3 × excess	Reaction 1: InGP → Tryp (units/ml)	Reaction 2: Ind → Tryp (units/ml)	Reaction 3: InGP → Ind (units/ml)	$\dfrac{\text{Reaction 1}}{\text{Reaction 2}}$	$\dfrac{\text{Reaction 3}}{\text{Reaction 2}}$
Sal. α	*Sal. β₂*	166	434	3·8	38·2	0·88
Sal. α	*E. coli β₂*	172	428	3·5	40·2	0·82
E. coli α	*E. coli β₂*	387	830	19	46·6	2·3
E. coli α	*Sal. β₂*	308	550	16	56·0	2·9
Sal. β₂	*Sal. α*	330	800	9·7	41·3	1·2
Sal. β₂	*E. coli α*	322	700	21·6	46·0	3·1
E. coli β₂	*E. coli α*	415	780	27·0	53·2	3·5
E. coli β₂	*Sal. α*	389	695	9·6	56·0	1·4

* Rates in all reactions compared to reaction 2 (indole → tryptophan), which is set at 100. InGP, Indole 3-glycerol phosphate; Ind, indole; Tryp, tryptophan.

4. DISCUSSION

Although each subunit of tryptophan synthetase is capable of carrying out one specific step in the conversion of InGP to tryptophan, optimal activity is only possible when it is associated with its complementary subunit in an $\alpha_2\beta_2$ complex (Yanofsky & Crawford, 1972; Crawford, 1975a, b). Each of these steps is a sub-reaction in the physiologically significant reaction InGP → tryptophan, and they are carried out sequentially by the complex. Thus the functionally significant regions of each subunit include not only the catalytic active sites but also those regions involved in subunit association. An important question to consider in studying the events leading to the divergence of bacterial tryptophan synthetases is whether these regions have undergone changes and, if so, what these changes have been. The present investigation concerns itself with this question.

By sedimentation studies of homologous and heterologous mixtures of α and β_2 subunits of tryptophan synthetases from two closely related species of Enterobacteriaceae, *E. coli* and *S. typhimurium*, we have been able to show that although association between heterologous subunits is quite good, as was already known (Balbinder, 1964; Murphy & Mills, 1969; Rocha *et al.* 1972), some rather interesting differences could be detected between both heterologous combinations, as well as between these and the homologous ones. We find it rather striking that while the association and sedimentation behaviour of the homologous *E. coli* α + *E. coli* β_2 subunit combination is practically identical to that of the *S. typhimurium* α + *S. typhimurium* β_2, the behaviour of the subunits in heterologous mixtures differed so sharply from these and even more sharply from each other, with the *E. coli* α + *S. typhimurium* β_2 showing a much weaker tendency to associate while in the reciprocal combination of *S. typhimurium* α + *E. coli* β_2 association was very strong. Murphy & Mills (1969) have studied the binding affinities of α subunits from *E. coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Enterobacter aerogenes* and *Serratia marcescens* for the β_2 subunit from *E. coli*. Using activation of the β_2 subunit in the indole $\rightarrow$ tryptophan reaction as the criterion, these authors found similar but slightly varying stimulating abilities for all of the α subunits. Among their observations, they reported that the α subunit from *S. typhimurium* binds the β_2 subunit of *E. coli* more tightly than the homologous α subunit of *E. coli* does. Our studies, which employed a completely different method, agree with this finding. Rocha *et al.* (1972) using the same general approach as Murphy & Mills (above) measured the association constants of β_2 subunits from the same bacterial species used by the latter authors, for the α subunit of *E. coli*. Among their findings is the observation that *S. typhimurium* β_2 has a lower association constant for binding to *E. coli* α than does the *E. coli* β_2 subunit, also in agreement with our results. The authors of these studies concluded that on the main, subunit binding sites on both the α and β components have been conserved in the course of evolution. Murphy & Mills (1969) further suggested that 'compensatory' mutations, i.e. changes in one subunit followed by compensatory changes in the complementary subunit, probably did not take place in the evolution of the Enterobacteriaceae. Our results, while agreeing with the conclusion that subunit binding sites of tryptophan synthetase components have been for the most part conserved during evolution, do indicate that compensatory changes also did take place. This is clearly suggested by the finding that association of α and β_2 is equal for homologous mixtures of *E. coli* and *S. typhimurium* subunits, but the degree of association in these mixtures is intermediate to that of the two heterologous combinations, as we have already described. Thus a degree of binding between tryptophan synthetase subunits which is neither too tight nor too loose appears to have a selective advantage and it is this strength of binding that has been retained during evolution. A mutation in the structural gene for the α subunit of *E. coli*, for example, leading to the substitution of an amino acid in its binding site for β_2 would confer a selective disadvantage to bacteria carrying it, but if the proper strength of binding were restored either by reversion to wild

type, a secondary mutation in the same gene leading to a second amino acid substitution in the same subunit, or a 'compensatory' mutation in the structural gene for the β subunit, the selective disadvantage would disappear.

We have insufficient data at this time to determine how frequently compensatory mutations might occur in the evolutionary divergence of bacterial tryptophan synthetases. It is interesting in this regard that a comparison of association constants for the binding between *E. coli* α and β_2 and complementary subunits from other bacterial sources shows that the α subunit of *Enterobacter aerogenes* resembles the one from *S. typhimurium* in having a higher association constant for the β_2 subunit of *E. coli* than does the α subunit of *E. coli* (Murphy & Mills, 1969). Also, the β_2 subunit of *E. aerogenes* resembles that of *S. typhimurium* in having a lower association constant for *E. coli* α than that of *E. coli* β_2. These parallels suggest that compensatory mutations could have occurred in the divergence of *E. aerogenes*, as in that of *S. typhimurium* from a common ancestor. Sedimentation experiments with the *E. aerogenes* α and β_2 subunits would be highly desirable to explore this question further. The entire amino acid sequences of the α subunits of *E. coli* (Guest *et al.* 1967), *S. typhimurium* (Li & Yanofsky, 1973 *a*) and *E. aerogenes* (Li & Yanofsky, 1973 *b*) are known. The α polypeptide chain of *S. typhimurium* differs from that of *E. coli* in 44 out of 268 amino acid residues, and that of *E. aerogenes* is different from the *E. coli* α chain in 39 residues. In an overall comparison, 79 % of the residues in the three α chains are identical. From the minimum base differences calculated on the basis of the amino acid difference data, Li & Yanofsky (1973 *b*) have determined that these three bacterial species diverged from a common ancestor within a relatively short period from each other, with *S. typhimurium* diverging a little earlier than the other two.

The effect of temperature in stabilizing the $\alpha_2\beta_2$ complex when this is subjected to an increase in pressure (centrifugation at higher speeds) was interpreted as an indication that hydrophobic bonding was an important factor in subunit association (DiCamelli *et al.* 1973). If this is the case, the tighter association between *S. typhimurium* α + *E. coli* β_2 as compared to *E. coli* α + *E. coli* β_2 would mean that the *S. typhimurium* α subunit has more hydrophobic amino acid residues than its *E. coli* counterpart, or that certain stretches of the *S. typhimurium* α chain contain more hydrophobic amino acid residues than the same stretches in the *E. coli* α chain. A comparison of the amino acid sequences of the two α chains (Li & Yanofsky, 1973 *b*) shows that at eight positions the *S. typhimurium* chain contains residues which are significantly more hydrophobic than those found at the corresponding position,* in *E. coli* and at five positions it has residues that are less hydrophobic than those found in *E. coli*.† Furthermore, in the stretch between amino acid residues 90 and 120, *S. typhimurium* has 17 hydrophobic residues vs. 15 in *E. coli* while there are no clear-cut changes in content of hydrophobic amino acid residues in the rest of the molecule. These differences in the content of

* Giving the number of the amino acid residue, and the *E. coli* residue first, these are: 108 (Asn-Ser), 109 (Lys-Pro), 194 (Asn-His), 204 (Asn-His), 221 (Lys-Val), 245 (Asn-Leu), 247 (Glu-Ser), 260 (Gln-Ser).

† These are: 6 (Ser-Asn), 68 (Thr-Asn), 198 (Ala-Glu), 244 (His-Asn), 254 (Ala-Glu).

hydrophobic amino acids are in line with expectations. A similar comparison between the *E. coli* and *E. aerogenes* α chains shows that the latter has six more hydrophobic residues than the former. Unfortunately there are no data at this time on amino acid differences between β subunits. One prediction of our hypothesis of compensatory mutations is that the β subunits of *S. typhimurium* and probably *E. aerogenes* will contain fewer hydrophobic amino acid residues than the β chain of *E. coli*, or that certain stretches of the *E. coli* β chain will be richer in non-polar amino acid residues than the corresponding stretches in the β chains of the other two species.

REFERENCES

BALBINDER, E. (1964). Intergeneric complementation between A and B components of bacterial tryptophan synthetases. *Biochemical and Biophysical Research Communications* **17**, 770–774.

CRAWFORD, I. P. (1975*a*). Tryptophan synthetase. Chapter 6 in *Subunit Enzymes; Structure and Function* (ed. K. Ebner). M. Dekker.

CRAWFORD, I. P. (1975*b*). Gene rearrangements in the evolution of the tryptophan synthetic pathway. *Bacteriological Reviews* **39**, 87–120.

CREIGHTON, T. E. & YANOFSKY, C. 1966. Association of the α and β_2 subunits of the tryptophan synthetase of *Escherichia coli*. *Journal of Biological Chemistry* **241**, 980–990.

DICAMELLI, R. F., BALBINDER, E. & LEBOWITZ, J. (1973). Pressure effects on the association of the α and β_2 subunits of tryptophan synthetase from *E. coli* and *S. typhimurium*. *Archives of Biochemistry and Biophysics* **155**, 315–324.

GOLDBERG, M. E., CREIGHTON, T. E., BALDWIN, R. L. & YANOFSKY, C. (1966). Subunit structure of the tryptophan synthetase of *Escherichia coli*. *Journal of Molecular Biology* **21**, 71–82.

GUEST, J. R., DRAPEAU, G. R., CARLTON, B. C. & YANOFSKY, C. (1967). The amino acid sequence of the A protein (α subunit) of the tryptophan synthetase of *E. coli*. *Journal of Biological Chemistry* **242**, 5442–5446.

LI, S., DENNEY, R. M. & YANOFSKY, C. (1973). Nucleotide sequence divergence in the α chain structural genes of tryptophan synthetase from *E. coli*, *S. typhimurium* and *A. aerogenes*. *Proceedings of the National Academy of Sciences, United States* **70**, 1112–1116.

LI, S. & YANOFSKY, C. (1972). Amino acid sequences of fifty residues from the amino termini of the tryptophan synthetase α chains of several enterobacteria. *Journal of Biological Chemistry* **247**, 1031–1037.

LI, S. & YANOFSKY, C. (1973*a*). Amino acid sequence studies with the tryptophan synthetase α chain of *S. typhimurium*. *Journal of Biological Chemistry* **248**, 1830–1836.

LI, S. & YANOFSKY, C. (1973*b*). Amino acid sequence studies with the tryptophan synthetase α chain of *A. aerogenes*. *Journal of Biological Chemistry* **248**, 1837–1843.

MURPHY, T. M. & MILLS, S. E. (1969). Immunochemical and enzymatic comparisons of the tryptophan synthetase α subunits from five species of *Enterobacteriaceae*. *Journal of Bacteriology* **97**, 1310–1320.

ROCHA, V., CRAWFORD, I. P. & MILLS, S. E. (1972). Comparative immunological and enzymatic study of the tryptophan synthetase β_2 subunit in the *Enterobacteriaceae*. *Journal of Bacteriology* **111**, 163–168.

SMITH, O. H. & YANOFSKY, C. (1962). Enzymes involved in the biosynthesis of tryptophan. In *Methods in Enzymology*, vol. v (ed. S. P. Colowick and N. O. Kaplan), pp. 794–806. New York: Academic Press.

SMITH, O. H. & YANOFSKY, C. (1963). Intermediates in the biosynthesis of tryptophan. *Methods in Enzymology* **6**, 590–597.

YANOFSKY, C. & CRAWFORD, I. P. (1972). Tryptophan synthetase. In *The Enzymes*, 3rd ed., vol. VII (ed. Paul D. Boyer), pp. 1–31. Academic Press.

YANOFSKY, C. & RACHMELER, M. (1958). The exclusion of free indole as an intermediate in the biosynthesis of tryptophan in *Neurospora crassa*. *Biochemica and Biophysica Acta* **28**, 640–641.