INTERGENERIC COMPLEMENTATION BETWEEN A AND B COMPONENTS OF BACTERIAL TRYPTOPHAN SYNTHETASES*

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The enzyme tryptophan synthetase of various microorganisms can catalyze the following three reactions (Yanofsky, 1960):

Reaction 1) indole-3-glycerol phosphate + L-serine → L-tryptophan + triose phosphate

Reaction 2) indole + L-serine → L-tryptophan

Reaction 3) indole-3-glycerol phosphate ⇄ indole + triose phosphate.

This enzyme consists of two dissociable non-identical protein components, A and B, in Escherichia coli (Crawford and Yanofsky, 1958) and other Enterobacteriaceae such as Salmonella typhimurium and Serratia marcescens (Balbinder, 1962a, b). In an earlier note (Balbinder, 1962b) it was reported that enzymatic activity was obtained in heterologous mixtures of A and B components, although immunological tests indicated the existence of structural differences among the different A components. In these early experiments only reaction 2 was measured. We wish to present at this time data confirming, and extending the earlier observations to include immunological tests on both the A and B proteins, cross-complementation in reaction 1 and relative affinities of different A proteins for different B's.

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Experimental:

As sources of A and B components, crude extracts of mutants lacking immunologically detectable protein as well as enzymatic activity for the complementary component (CRM^-) were used. For E. coli mutants B-4 and A-2 of C. Yanofsky's collection, and for S. typhimurium mutants try D-55 and try C-44 of M. Demerec's collection were the sources of the A and the B components respectively. A mutant of S. marcescens, strain HY, which had no detectable B-CRM was the source of Serratia-A. No Serratia-B protein free of A was available for these experiments. E. coli and S. typhimurium were cultured according to the procedure of Smith and Yanofsky (1962), S. marcescens as described by Belser and Bunting (1956). The assays for reactions 1 and 2 are those of Smith and Yanofsky (1962). Reaction 1 was additionally assayed by the tryptophanase method of De Moss (1962). Antisera against components A and B of E. coli were obtained through the courtesy of Drs. C. Yanofsky and I. P. Crawford respectively. Those against Salmonella-A and B were obtained by the author by injecting partially purified preparations of each component into rabbits.

The relevant observations can be summarized as follows:

a) Enzyme neutralization tests utilizing anti-Salmonella-A disclosed no differences among all three A components. When anti-Escherichia-A was used, however, differences in the extent of neutralization were observed. The titer of this antiserum against Salmonella-A was about 30%, and against Serratia at most 5%, of that against E. coli.

b) Enzyme neutralization tests with both anti-Salmonella-B and anti-Escherichia-B failed to show differences among all three B components. However, precipitation in gel by the Ouchterlony technique (Ouchterlony, 1949) with both antisera disclosed differences between Serratia-B on one hand and the B components of S. typhimurium and E. coli on the other, but no differences between the latter two were demonstrable.

c) In Reaction 2, the activities observed for the same crude

extract of A component were the same regardless of the source of complementing B component (Table 1).

d) Activity in Reaction 1 can be obtained in heterologous mixtures as shown in Table 2 for a Serratia-A + Salmonella-B combination.

TABLE 1

Activities (units/ml) in reaction 2 (ind. → tryp.) of crude extracts of A components of S. typhimurium and S. marcescens tryptophan synthetase, determined against saturating amounts (10-fold excess) of B components from E. coli and S. typhimurium.

Source of Component A	Component B: None added	From S. typhimurium	From E. coli	Average and Range
Salmonella typhimurium	0	997	1100	1048 ± 52
Serratia marcescens	0	47	59	53 ± 6

TABLE 2

Activities (units/ml) of Salmonella tryptophan synthetase (both components), and of heterologous Serratia A - Salmonella B mixtures in reactions 1 and 2.

Extract	Reaction 1: IGP. → tryp.	Reaction 2: Ind. → tryp.	$\frac{\text{Reaction 1}}{\text{Reaction 2}} \times 100$
Salmonella (wild type)	56	160	35
Salmonella-A*	137	358	38.2
Serratia-A*	32	49	65.3
Salmonella-B**	120	304	39.5

* In presence of a tenfold excess of component B from Salmonella.

** In presence of a tenfold excess of component A from Salmonella.

Note that Serratia-A in the presence of Salmonella-B shows a higher $\frac{\text{reaction 1}}{\text{reaction 2}}$ ratio than any of the homologous combinations studied. Whether this reflects a property of the Serratia-Salmonella combination or of the Serratia enzyme itself is not clear at present. The homologous Salmonella-Salmonella combinations show ratios of $\frac{\text{reaction 1}}{\text{reaction 2}}$ not essentially different from those reported for the Neurospora crassa and E. coli tryptophan synthetases (Yanofsky, 1960).

e) The relative affinities of the different A's for various B proteins were studied in saturation experiments (Yanofsky and Crawford, 1958). Representative results are shown in Fig. 1.

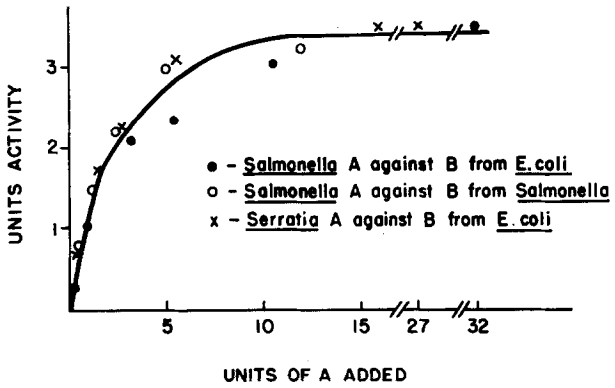

Fig. 1. Saturation of B. components of S. typhimurium and E. coli with different A components. Activity measured in Reaction 2 (ind. → tryp.). To crude extracts containing 3.5 units of B component activity, varying amounts of crude extracts of the different A components were added as indicated in the graph.

Data of this nature show no significant differences in the affinities of the different A components for the B components employed. Early experiments (Balbinder, 1962b) were erroneously interpreted indicating differences in the extent of complementation.

The extent and nature of the structural differences between the different enzyme components is not yet known. Preliminary peptide analyses for the A component of S. typhimurium indicate differences in several peptides with respect to that of E. coli K-12, (D. Helinski, private communication). No information is yet available for S. marcescens-A or any of the B components.

From our results it is clear that, in the combinations we have studied, the differences in structure between the various A and B proteins are not sufficient to interfere with their effective interactions in restoring enzymatic activity. In contrast, in similar studies utilizing

more distantly related organisms, less efficient complementation was observed. This was the case in heterologous mixtures between components from E. coli, S. typhimurium and S. marcescens on one hand and the complementary components of Aeromonas formicans (D. K. Melhorn and I. Crawford, private communication), Bacillus subtilis (A. Keller, private communication) and the blue-green alga Anabaena variabilis (K. Sakaguchi and D. M. Bonner, private communication) on the other. The structural factors influencing interactions between A and B proteins and the possibility of employing "hybrid" enzymes as taxonomic tools are the subjects of future investigations.

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