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Comparison of the Tryptophan Synthetase α Subunits of Several Species of *Enterobacteriaceae*

T. E. CREIGHTON, D. R. HELINSKI,[1] R. L. SOMERVILLE, AND C. YANOFSKY

Department of Biological Sciences, Stanford University, Stanford, California, and Department of Biochemistry, The University of Michigan, Ann Arbor, Michigan

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ABSTRACT

CREIGHTON, T. E. (Stanford University, Stanford), D. R. HELINSKI, R. L. SOMERVILLE, AND C. YANOFSKY. Comparison of the tryptophan synthetase α subunits of several species of *Enterobacteriaceae*. J. Bacteriol. **91**:1819–1826. 1966.—The tryptophan synthetase α subunits of *Escherichia coli* K-12, *E. coli* B, *Shigella dysenteriae*, *Salmonella typhimurium*, and *Aerobacter aerogenes* have been purified and their structures compared. Each of these α subunits exhibits a sedimentation coefficient of about 2.7*S*. Peptide patterns of trypsin plus chymotrypsin digests of the α subunits have indicated that all of the α subunits have peptide regions in common. The patterns of *E. coli* K-12, *E. coli* B, and *S. dysenteriae* α subunits appear to be nearly identical, whereas the α subunits from *S. typhimurium* and *A. aerogenes* differ from those of *E. coli* and from each other. It has also been shown that the *E. coli* structural gene for the α subunit is translated identically in *E. coli* and *S. typhimurium*.

Evolutionary processes which have maintained a high degree of basic biochemical unity would be expected to result in truly homologous, as opposed to analogous, genes in related species. Evidence for the existence of genetic homology has been obtained by comparing the primary genetic material, deoxyribonucleic acid (DNA), from various bacterial species (25, 30) and, perhaps more significantly, by comparing protein primary structures (13, 20, 23, 25). Investigations of primary structure similarities will undoubtedly also contribute to our understanding of the relationships between structure and function. Thus, regions of a protein which vary considerably in amino acid sequence in different species are likely to have a relatively limited role in the biological activity of that protein. On the other hand, regions which have remained largely unaltered may have a direct role in the function of the protein, as has been suggested by several investigators (12, 26, 28). When combined with studies of mutationally altered proteins (15, 38), such comparisons should yield valuable information on structure-function relationships.

For these reasons we have initiated a study of the structure of the tryptophan synthetase [L-serine hydro-lyase (adding indole), EC 4.2.1.20] of several bacterial species (35). The diversity of properties that this enzyme possesses in several microbial species (3) adds considerable interest to a study of its evolutionary variation. In this paper, we report the results of a comparative study of the tryptophan synthetase α subunits (formerly designated A proteins) from several enterobacteria and their hybrids.

[1] Present address: Department of Biology, University of California, San Diego, La Jolla.

MATERIALS AND METHODS

The α subunits were identified by their ability to catalyze the conversion of indole plus serine to tryptophan (32) in the presence of the *Escherichia coli* K-12 tryptophan synthetase β_2 subunit (formerly designated the B protein) and the coenzyme pyridoxal phosphate. The α subunits were obtained from extracts of the following bacteria: *E. coli* K-12 (5), *E. coli* B (9), an *E. coli* B-*Shigella dysenteriae* hybrid carrying the A gene (the structural gene for the α subunit) from *S. dysenteriae* (9), *Salmonella typhimurium* (1), a *S. typhimurium* strain containing the A gene from *E. coli* K-12 (see following), and *Aerobacter aerogenes*. In each case, a mutant strain with a biochemical block early in the tryptophan pathway was employed so that high levels of the tryptophan biosynthetic enzymes were produced under derepression conditions.

The *E. coli* K-12 A gene was transferred to *S. typhimurium* on an F′ episome (10) by use of methods previously described (33). The *E. coli* K-12 donor

carried the episomal markers *cys*$^+$ and A46 [mutationally altered A gene producing an inactive α subunit (17)] and the chromosomal markers *Lp*^{s}, *his*$^-$, *pro*$^-$, *cys*$^-$, *A46*, *str-r*. *S. typhimurium* 8 CT1 (24), in which the entire *cys-try* region is deleted, was used as the recipient. The desired hybrids were isolated at low frequency (about 1 recombinant per 10^8 donor bacteria) by selecting for *cys*$^+$, *try*$^+$ on minimal medium (33*a*) plus leucine and indole. The hybrids were *ind*$^-$, accumulated indoleglycerol, and transferred F′ A46 to *E. coli* and *S. typhimurium* at frequencies of 10^{-3} and 10^{-5}, respectively.

Since the *S. dysenteriae* A gene was transduced into an *E. coli* B strain which lacked the entire A gene as a result of a genetic deletion, the *E. coli* B-*S. dysenteriae* hybrid presumably forms the α subunit specified by the *S. dysenteriae* A gene.

The α subunits were purified as previously described (16) or by slightly modified procedures.

Sedimentation velocity measurements were performed with a Beckman/Spinco model E analytical ultracentrifuge at 20 C in a filled-Epon, double-sector, synthetic-boundary cell at 50,740 rev/min. The solvent was 0.03 M potassium phosphate buffer (*p*H 7.0). Measurements from photographs were made with a two-dimensional Gaertner comparator.

Proteolytic digestion of the α subunits and two-dimensional separation and staining of peptides were as previously described by Helinski and Yanofsky (14). After two-dimensional separation, the areas containing selected peptides were cut out, and the peptides were eluted with 0.1 M NH_4OH. In some cases, the peptides were reacted with ninhydrin before elution from the paper. The amino acid content of peptides was determined with a Beckman/Spinco amino acid analyzer after hydrolysis of the peptide at 105 C for 48 hr in 5.7 N HCl in sealed evacuated tubes.

RESULTS

The α subunit from *E. coli* K-12 has previously been shown to have a sedimentation coefficient ($S_{20,w}$) of 2.7*S* which was relatively independent of the protein concentration (16). The sedimentation coefficients of the other α subunits studied were found to be essentially identical to 2.7*S* (Fig. 1). In all cases, the α subunits sedimented with a single symmetrical boundary.

Two-dimensional separation of peptides of proteolytic digests of the *E. coli* K-12 α subunit has been shown to produce very distinctive and reproducible peptide patterns (14). This technique has proven to be useful in examining evolutionary relationships of homologous proteins, especially those of the hemoglobins (18, 40). Accordingly, the peptides of trypsin plus chymotrypsin digests of purified α subunits were separated and stained with ninhydrin and reagents specific for arginine- and tyrosine-containing peptides (Fig. 2 and 3). Peptide patterns of trypsin plus chymotrypsin digests were chosen

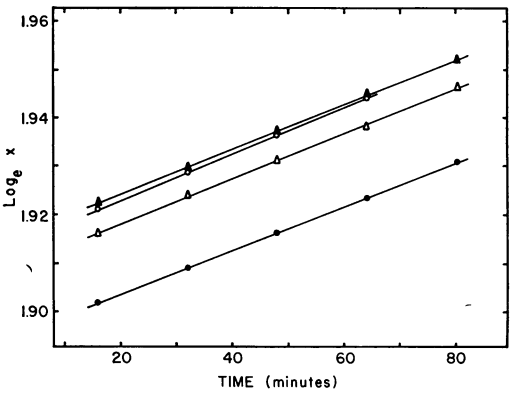

FIG. 1. *Plot of the logarithm of the distance of the boundary from the axis of rotation (x) as a function of time during sedimentation at 50,740 rev/min of the various tryptophan synthetase α subunits. The α subunits were obtained from Escherichia coli B (○), Shigella dysenteriae (●), Salmonella typhimurium (△), and Aerobacter aerogenes (▲). The values of $S_{20,w}$ calculated were 2.78S for E. coli B, 2.69S for S dysenteriae, 2.71S for S. typhimurium, and 2.72S for A. aerogenes. The protein concentrations were 6.2, 3.4, 7.5, and 7 mg/ml, respectively.*

for comparison because this combination of proteolytic enzymes digests almost the entire *E. coli* K-12 α subunit into soluble peptides (J. Guest, B. Carlton, and C. Yanofsky, *unpublished data*). In comparing peptide patterns, peptides which were located at identical positions and exhibited the same reactions to the specific amino acid stains were considered identical.

Comparison of the peptide patterns of the various α subunits indicates that those of the *E. coli* K-12 and *E. coli* B α subunits are essentially identical (Fig. 3a), whereas the pattern obtained with the *S. dysenteriae* α subunit differs from the *E. coli* patterns in about three peptide spots (Fig. 3b). In contrast, the *S. typhimurium* and *A. aerogenes* α subunit peptide patterns differ quite markedly from those of *E. coli* and from each other. Of the 38 to 41 major peptides detected in a trypsin plus chymotrypsin peptide pattern, the peptide patterns of the *S. typhimurium* and *A. aerogenes* α subunits have 21 and 18 peptide spots in common with that of the *E. coli* K-12 α subunit, respectively (Fig. 3c, d). There are approximately 19 such spots in common between the *S. typhimurium* and *A. aerogenes* α subunit peptide patterns (Fig. 3e). Furthermore, there are 13 ninhydrin-positive spots which are present in all of the peptide patterns (Fig. 3f). However, several of these spots represent free amino acids and, thus, are of less importance in peptide pattern comparisons. As indicated in Fig. 3, the total

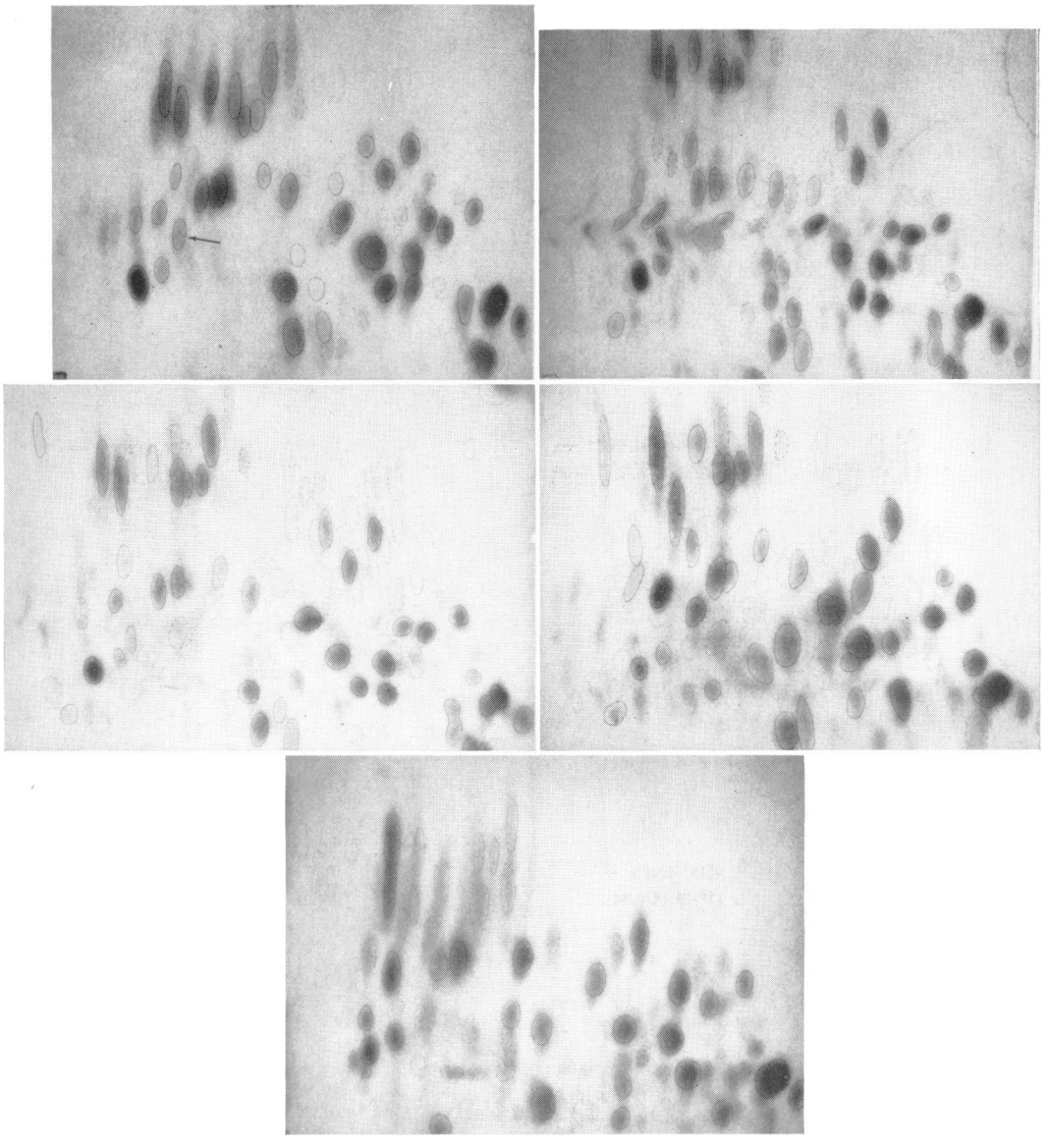

FIG. 2. *Ninhydrin-stained trypsin plus chymotrypsin peptide patterns of the tryptophan synthetase α subunits from Escherichia coli K-12 (top left), E. coli B (top right), Shigella dysenteriae (middle left), Salmonella typhimurium (middle right), and Aerobacter aerogenes (bottom). Chromatography was carried out in the vertical direction and electrophoresis in the horizontal direction. Major peptide spots are circled with a solid line and minor peptide spots with a dashed line. The arrow in the peptide pattern of the E. coli K-12 α subunit indicates the position of peptide TP3C1.*

number of peptide spots is very nearly the same in all of the peptide patterns.

Specific amino acid stains indicated probable homology between several nonidentical peptides at related positions on the peptide patterns. Examples of this are the two pairs of tyrosine-containing peptides at the same electrophoretic, but slightly altered chromatographic, positions in the *E. coli* K-12 and *S. typhimurium* α subunit peptide patterns (Fig. 3c).

Peptide TP3C1 from the *E. coli* K-12 α subunit has been studied extensively in mutation and reversion studies (4), and thus its composition in the other α subunits is of particular interest. Peptides

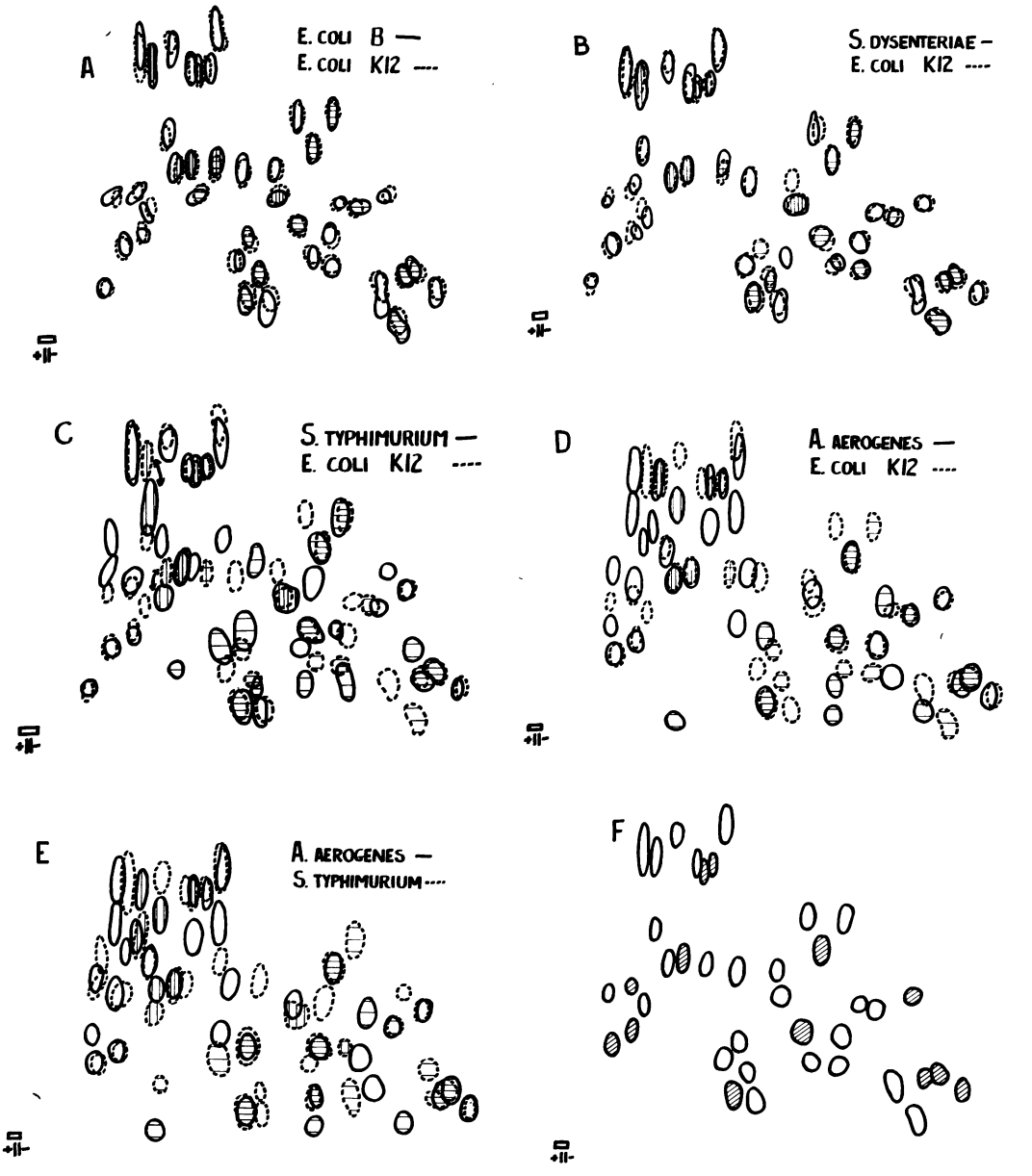

FIG. 3. *Schematic comparisons of the major peptide spots of the peptide patterns of the various α subunits. Vertical lines indicate spots which stained positive for tyrosine, and horizontal lines indicate spots which stained positive for arginine. Where overlapping peptides gave the same color reaction, the spacing between vertical or horizontal lines was halved. The peptide patterns compared are those of the α subunits from (a) Escherichia coli K-12 and E. coli B, (b) Shigella dysenteriae and E. coli K-12, (c) Salmonella typhimurium and E. coli K-12, (d) Aerobacter aerogenes and E. coli K-12, (e) S. typhimurium and A. aerogenes. The arrows in (c) indicate suggested possible related peptides in the E. coli K-12 and S. typhimurium α subunits. The peptide pattern of the E. coli K-12 α subunit is represented in (f) with cross-hatching to indicate those peptide spots which appear to be common to all of the α subunit peptide patterns.*

were found at a position corresponding to TP3C1 in the patterns of the *E. coli* B and *S. dysenteriae* α subunits. Upon isolation, the amino acid content of each peptide was found to be identical to that of the TP3C1 peptide of the *E. coli* K-12 α subunit (Table 1). No peptide corresponding to TP3C1 was apparent in the peptide patterns of the *S. typhimurium* and *A. aerogenes* α subunits.

TABLE 1. *Analysis of peptide TP3C1 from various sources*

Source of peptide TP3C1	Amino acid composition*						
	Asp†	Glu†	Pro	Gly	Ala	Leu	Phe
Escherichia coli K-12.....	1	1	2	1	2	1	1
E. coli B.....	0.48‡	1.2	1.9	1.2	1.8	1.1	.84
Shigella dysenteriae.....	0.93	1.07	2.00	1.08	2.00	0.98	0.91

* Asp = aspartic acid; Glu = glutamic acid; Pro = proline; Gly = glycine; Ala = alanine; Leu = leucine; Phe = phenylalanine.

† Not determined whether present as acid or amide.

‡ Value low because aspartic acid is N-terminal and this peptide was treated with ninhydrin.

The question arises as to what extent the results of comparing protein structures are affected by possible differences in translation of the corresponding genes. To answer this question in one instance, the peptide pattern of the α subunit specified by the *E. coli* K-12 A gene (containing the A46 mutation) in *S. typhimurium* cytoplasm (Fig. 4) was compared with the pattern of the wild-type *E. coli* α subunit (Fig. 2). Although the peptide patterns are not identical because of the A46 mutational alteration and slight differences in chromatographic conditions, it is clear that none of the peptide spots characteristic of the *S. typhimurium* α subunit are present. The *E. coli* α subunit gives the same pattern under these chromatographic conditions. It is also to be noted that the mutational alteration characteristic of the A46 α subunit in *E. coli* K-12 was also evident when this subunit was synthesized in *S. typhimurium*. This result indicates that the *E. coli* A gene is translated identically in *E. coli* and *S. typhimurium*.

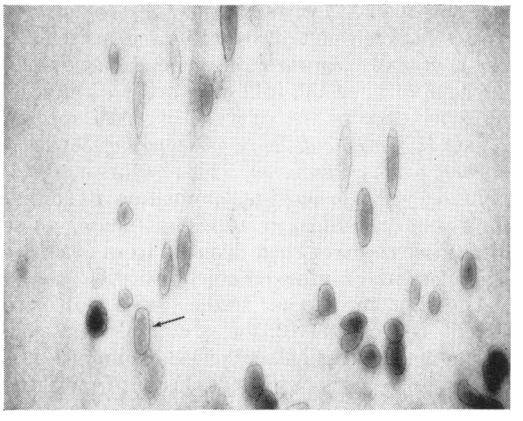

FIG. 4. *Ninhydrin-stained trypsin plus chymotrypsin peptide pattern of the Escherichia coli K-12 tryptophan synthetase α subunit synthesized in Salmonella typhimurium. Chromatography was carried out in the vertical direction and electrophoresis in the horizontal direction. Major peptide spots are circled with a solid line and minor spots with a dashed line. The arrow indicates the position of the peptide TP3C1 of the A46 mutant α subunit. In this peptide, glutamic acid replaces a glycine residue of the wild-type α subunit (7).*

DISCUSSION

The tryptophan synthetase α subunits of the enterobacteria studied appear to be truly homologous proteins. They are all very similar in size, as estimated from their sedimentation behavior and the number of peptides obtained after digestion with trypsin plus chymotrypsin. In addition, their peptide patterns suggest that there are many homologous peptide regions within the α subunits. Although the *S. typhimurium* and *A. aerogenes* α subunit peptide patterns differ from each other and from those of the *E. coli* α subunit, comparison of any two of the peptide patterns indicates that they have in common a minimum of nearly half the peptide spots.

In analyzing data of this type, certain reservations must be kept in mind. Peptides appearing at the same position on the peptide patterns need not be identical. On the other hand, peptide pattern comparisons may exaggerate the extent of differences between homologous proteins, since there is ample evidence that peptides differing by only a single amino acid residue appear at different positions (19, 37). Another possible weakness of proteolytic digest comparisons is that certain regions of a protein may not be examined because of resistance to proteolytic digestion. However, primary structure studies with the *E. coli* K-12 α subunit (Guest, Carlton, and Yanofsky, *unpublished data*) suggest that most of the susceptible bonds are attacked by the combined action of trypsin plus chymotrypsin. In spite of these reservations, peptide pattern comparisons can yield valuable information on relative degrees of primary sequence homology between presumed related proteins (18, 40).

Homology of the genetic material of different enterobacteria has been amply demonstrated by a variety of methods. On a gross scale, the arrangement of genes on the chromosomes of *E.*

coli and *S. typhimurium* is remarkably similar (29). Intergeneric recombination between these organisms has been demonstrated, and the observed frequencies have been interpreted in terms of homology of gene structure. On the latter basis, *E. coli* K-12 and *E. coli* B appear to be nearly completely homologous, whereas *E. coli* and *S. dysenteriae* are less so (21, 22). *E. coli* and *S. typhimurium* give markedly low recombination values (8, 39), suggesting dissimilarities at the fine-structure level. In particular, the recombination frequencies detected in the region of the tryptophan biosynthetic genes in transduction crosses between *E. coli* and *S. typhimurium* were less than 1% of the frequencies obtained in crosses between mutants of the same species (7). The degree of hybridization of nucleic acids (25, 30) has indicated the following order of increasing divergence of the relevant bacterial species: *E. coli* K-12, *E. coli* B, *S. dysenteriae*, *S. typhimurium*, and *A. aerogenes*. This result is in agreement with the genetic recombination data that are available. Similar relationships among these enterobacteria have been deduced from extensive examinations of other characteristics.

Although the above studies have not been directly concerned with the structural genes specifying the tryptophan synthetase α subunits, it is of interest to compare the conclusions from those studies with those arrived at in the present investigation. Any conclusion from the present investigation concerning the relative homology of the various α subunit structural genes must depend upon the fidelity with which the primary structure of a protein represents the nucleotide sequence of its structural gene, i.e., the universality of the genetic code. The results of this investigation have shown that the genetic code is probably identical in *E. coli* and *S. typhimurium*, and a similar conclusion has been reached in other cases on the basis of other studies in vitro (6) and in vivo (31). However, the nature of the degeneracy of the genetic code (27) makes it likely that the homology between proteins may be somewhat greater than the homology between their structural genes. But if we make the assumption that the peptide patterns of the tryptophan synthetase α subunits are valid representations of their primary structure and that the primary structure is an accurate representation of the corresponding structural gene, it is clear that the relative degrees of similarity between the α subunits are qualitatively consistent with the relative degrees of genetic homology detected by other means. Of course, no quantitative comparisons can be made at this time of the degrees of homology estimated by different methods. However, the approach of studying the protein product of a single gene obviously complements other studies of genetic homology.

Mutation and reversion studies with the *E. coli* K-12 α subunit have shown that at least two other amino acids, serine and alanine, may replace the glycine of peptide TP3C1 with no detectable effect on the in vivo activity of tryptophan synthetase (4). It is also likely that glycine and alanine may replace one another at this position as a result of a single mutational event, i.e., a single nucleotide change (36). If the *E. coli* K-12, *E. coli* B, and *S. dysenteriae* α subunits are as similar as their peptide patterns indicate, the appearance of glycine in the peptide corresponding to TP3C1 in each case would indicate that a known possible "neutral" evolutionary event has *not* taken place. The absence of a peptide at the location of TP3C1 in the peptide patterns of the *S. typhimurium* and *A. aerogenes* α subunits suggests that the corresponding regions of these two α subunits have undergone some alteration.

The evolutionary study of proteins is also valuable for the information it may yield on the relation of protein structure to biological activity. The general interpretation of such comparative results is that the extent of evolutionary alteration of a protein segment is inversely proportional to its role in the protein's biological activity. The α subunit is somewhat unique in that it exhibits maximal enzyme activity only when it is complexed with the tryptophan synthetase β_2 subunit (5). Thus, the functionally significant regions of the α subunit include not only the catalytic active site (11), but also the region involved in combining with the β_2 subunit. In some respects the evolution of an α subunit should ideally be considered along with that of the homologous β_2 subunit. It is possible that certain evolutionary alterations of the α subunit would only be compatible with complementary alterations of the β_2 subunit. This would only be evident from an examination of both subunits. The activity of mixtures of intergenerically different α and β_2 subunits has been investigated with the tryptophan synthetase subunits of *E. coli* and *S. typhimurium* (2). The hybrid enzyme complexes were formed as readily as with the intrageneric pairs, and they were enzymatically active. In the present investigation, the α subunits studied were all active with the *E. coli* K-12 β_2 subunit in catalyzing the conversion of indole plus serine to tryptophan (32). This finding would suggest that the combining sites of the two subunits have been conserved during the evolutionary divergence of the bacterial species examined. In this regard, it should be noted that there are several peptides

in the peptide patterns which appear to be common to all of the α subunits studied. Whether these peptides are from regions of the α subunit that contribute to the active site or from regions essential for complexing with the β_2 subunit remains to be determined.

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