Interspecies Hybrid Tryptophan Synthase-Modified β2 Protein Formed from Separate Folding Regions of the β Monomer

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Escherichia coli and Serratia marcescens tryptophan synthase β2 protein (EC 4.2.1.20) was subjected to mild trypsin proteolysis. Two separate folding regions (domains) of the E. coli (EF1 and EF2) and the S. marcescens (SF1 and SF2) enzyme were shown to form interspecies hybrid reconstituted molecules [(EF1-SF2)2 and (SF1-EF2)2] and intraspecies reconstituted molecules [(EF1-EF2)2 and (SF1-SF2)2] with equal efficiency. The data suggest that structural regions, associated with β monomer assembly, exist somewhere on the domain fragments and that these regions are conserved.

Recent experiments by Högborg-Rai baud and Goldberg (6, 7) and by our group (13) indicate that the β2 protein of tryptophan synthase from enteric bacteria can be selectively proteolysed such that two nonoverlapping, independently folding peptide fragments (domains β-F1 and β-F2) can be recovered. It has been suggested for the β2 protein (7), and other proteins (4, 16), that domains represent intermediates that transiently exist during the biosynthesis and assembly of the protein from which they derive. Crawford et al. (3) have shown that for Escherichia coli, F1 comprises approximately the first two-thirds of the native protein including the N-terminus, while F2 contains the remaining third of the uncleaved molecule, including the C-terminus; a small interconnecting region is lost during proteolysis. More recently, we showed that the domains obtained from trypsinolysis of the Serratia marcescens β2 protein can immunochemically cross-react with antiserum prepared against the native β2 protein (13). Reconstitution experiments plus the apparent independent folding character of the β2 protein domain fragments imply that regions of interaction (binding sites) exist on the surface of these domain fragments. Our interest in the binding region aspect of this question derives from previous studies of the β2 protein which have clearly shown the enzyme to be a conserved gene product among the enteric bacteria (1, 11, 14). We have suggested that the strong conservation of the β2 protein is a consequence of significant binding responsibilities associated with enzyme catalysis (2, 10, 14, 15, 17). The possibility of constructing interspecies and interspecies reconstituted nicked β2 protein provides an approach to examining the relative structural similarity and conservation of the regions of the β monomer concerned with domain association; the results of such experiments are reported here.

E. coli A2/F′A2 was obtained from I. P. Crawford, and S. marcescens trpE7 was constructed in our laboratory as previously described (12). Tryptophan synthase β2 protein was isolated from E. coli by the procedure of Wilson and Crawford (18), and S. marcescens β2 protein was obtained by a modified E. coli procedure (12, 13). Protein was measured by the method of Lowry et al. using bovine serum albumin as standard (8).

E. coli and S. marcescens β2 protein was subjected to mild trypsin proteolysis using the procedure of Högborg-Rai baud and Goldberg (6, 13). The two domain fragments from E. coli (EF1 and EF2) and from S. marcescens (SF1 and SF2) were separated and isolated in pure form by urea-gel permeation chromatography (data not shown) (7, 13). The isolated domains were used for reassociation experiments where formation of reconstituted nicked β2 enzyme was monitored by the fluorescence properties of the “aqua” complex, an intermediate in the β2 protein catalytic reaction sequence (5, 6). The amplitude of fluorescence of the reconstituted Serratia complex is essentially equivalent to that of the E. coli complex; as with the E. coli enzyme, nicking reduces the fluorescence of the S. marcescens native enzyme to a small degree (7). Neither domain fragment alone has this fluorescence capability.

Initially, reconstitution experiments were performed using domain fragments obtained from the S. marcescens β2 protein (intraspecies reconstituted nicked β2 protein). The results of titrating a fixed amount of SF1 with varying amounts of SF2 are presented in Fig. 1A. The parallel experiment using isolated domain fragments from the E. coli enzyme yielded similar results and is described in Fig. 2A. It can be seen that maximum reconstitution occurs at a molar ratio of approximately 1 for both the S. marcescens
and *E. coli* titrations. In addition, the slopes of the two curves are similar (*S. marcescens* = 0.76; *E. coli* = 0.64).

After establishing that both the *S. marcescens* and *E. coli* $\beta_2$ enzymes could be reconstituted into nicked $\beta_2$ molecules, we attempted to construct reconstituted nicked $\beta_2$ molecules using domains from *S. marcescens* and *E. coli* $\beta_2$ protein (interspecies hybrid molecules). The results of titrating a fixed amount of SF1 with a varying amount of EF2 or, alternatively, a fixed amount of EF1 with a varying amount of SF2, are presented in Fig. 1B and 2B. As was the case in the interspecies reconstitution experiments, the interspecies hybrid reconstitution titration curves showed that maximum recombination occurs at a molar ratio of approximately 1 for both hybrid reconstituted molecules. Furthermore, slopes of the two hybrid molecule titration curves are similar in value (SF1 + EF2 = 0.76; EF1 + SF2 = 0.62).

The most important comparison is that between the intraspecies reconstitution curve and the interspecies hybrid titration curve. The molar saturation ratios (approximately 1) were very similar (see Fig. 1 and 2) for both sets of experiments. The slope values for SF1 + SF2 and SF1 + EF2 were identical at 0.76; those for EF1 + EF2 and EF1 + SF2 were very similar at 0.64 and 0.62, respectively. These data indicate that the *S. marcescens* and *E. coli* domain fragments are able to form very stable, stoichiometric complexes in either homologous or heterologous combinations.

These observations suggest that structural regions exist on both the small- and large-domain fragments of the two enzymes studied, which provide for association of the two domains to form reconstituted nicked $\beta_2$ protein molecules. Furthermore, these data argue strongly that the regions of the domain interactions are conserved in the $\beta_2$ protein from the two organisms examined. Selective pressure on $\beta$ monomer domains to maintain their mutually complementary molecular configuration may involve (i) pressure to conserve shape, ensuring that $\beta$ monomer assembly proceeds correctly allowing for proper dimerization to form the native $\beta_2$ protein, and (ii) the possibility of some aspect of the enzyme's active site overlapping with the F1-F2 binding site, placing strong selection pressure on maintenance of proper domain geometry because of the enzyme's catalytic characteristics. The fact that $\alpha$-subunit binding to the $\beta_2$ protects it from proteolysis (9) argues that the $\beta$ monomer cleavage site and perhaps the domain binding sites are associated with the enzyme's active site.

Differences in primary structure do exist between the *E. coli* and *S. marcescens* $\beta_2$ proteins,
as shown by comparative trypsin peptide pattern studies (1). Presumably they occur at nonfunctional sites; however, no comparative sequence data are currently available regarding this point.

Thus, it appears that surface binding sites (subunit, cofactor, and substrate types) plus internal binding sites associated with protein assembly may both operate to limit significant evolutionary divergence of the enteric bacteria β2 protein and perhaps other proteins that have domain-type protein architecture. Binding regions associated with protein assembly may have a very significant influence on the evolution of proteins. Systems like the β2 protein provide excellent models to examine this interesting question.

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LITERATURE CITED


