Subunit communication in the tryptophan synthase $\alpha_2\beta_2$ complex

Effects of $\beta$ subunit ligands on proteolytic cleavage of a flexible loop in the $\alpha$ subunit

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Received 8 January 1992

To probe the structural basis for ligand-mediated communication between the $\alpha$ and $\beta$ subunits in the tryptophan synthase $\alpha_2\beta_2$ complex, we have determined the effects of ligands of the $\alpha$ and $\beta$ subunits on proteolysis of a flexible loop in the $\alpha$ subunit. We find that addition of a ligand of the $\beta$ subunit (L-serine, o-tryptophan, or L-tryptophan) in combination with a ligand of the $\alpha$ subunit (o-glycerol 3-phosphate) almost completely prevents the tryptic cleavage of the $\alpha$ subunit loop. Thus, the binding of a ligand to the $\beta$-site affects the conformation of the $\alpha$ subunit 25–30 Å distant.

Tryptophan synthase; Subunit communication; Allosteric mechanism; Protein loop; Proteolysis; Ligand binding

1. INTRODUCTION

An important problem in the elucidation of the allosteric mechanism is the structural basis for ligand-mediated communication between topologically distinct binding sites. An ideal system for investigating this problem is the bacterial tryptophan synthase $\alpha_2\beta_2$ complex (EC 4.2.1.20) that catalyzes the final reactions in the biosynthesis of L-tryptophan [1–3]. Crystallographic studies show that the $\alpha$ and $\beta$ active sites are 25–30 Å apart and are connected by a tunnel [4]. Since ligands that bind at one active site influence the properties of the other site, the heteroligand sites communicate reciprocally over a distance of 25–30 Å [5–7]. These allosteric interactions result from ligand-induced conformational changes that are transmitted from one protomer to the other.

Tryptsin cleaves the tryptophan synthase $\alpha_2\beta_2$ complex at Arg-188 in the $\alpha$ subunit and produces two fragments termed $\alpha$-1 and $\alpha$-2 [8,9]. Arg-188 is located in a long, disordered loop in the $\alpha$ subunit (residues 179–192) that can not be seen in the crystal structure of the $\alpha_2\beta_2$ complex from Salmonella typhimurium [4,10]. Our finding that addition of a ligand of the $\alpha$ subunit decreases the rate of cleavage by trypsin suggest that ligand binding alters the conformation and flexibility of the loop [11]. An allosteric role for the $\alpha$ subunit loop is supported by the observation that the native $\alpha_2\beta_2$ complex is strongly inhibited by a ligand of the $\alpha$ subunit whereas the ‘nicked’ $\alpha_2\beta_2$ complex is desensitized to this inhibition [11].

2. MATERIALS AND METHODS

2.1. Enzymes, assays, and proteolysis

Tryptophan synthase $\alpha_2\beta_2$ complex from S. typhimurium was purified as described [12]. Solutions of the $\alpha_2\beta_2$ complex (1.6 mg/ml in 50 mM sodium N,N-bis(2-hydroxyethyl)glycine buffer containing 1 mM EDTA at pH 7.8) were treated at 22°C with 1 µg/ml TPCK-trypsin (Cooper Biomedical) in the presence or absence of ligands; reactions were stopped by addition of trypsin inhibitor [11]. Aliquots (5–10 µl) were assayed for activity in the synthesis of L-tryptophan from indole and L-serine in the presence or absence of 80 mM DL-o-glycerol 3-phosphate (Sigma) [13].

2.2. Gel electrophoresis and densitometry

Sodium dodecyl sulfate gel electrophoresis of proteins and staining with Coomassie blue R-350 utilized a Phast System (Pharmacia LKB Biotechnology) [11]. The Hoefer GS-360 Data System and GS-500 scanning densitometer were used to scan gels stained with Coomassie blue R-250. Areas of peaks ($\alpha$ subunit and $\alpha$-1 fragment) were obtained by Gaussian integration. The fractional cleavage is defined as the (area of $\alpha$ fragment)/ (area of $\alpha$ fragment + area of $\alpha$ subunit).

3. RESULTS

The present study asks whether ligands bound to the $\beta$-site communicate allosteric effects to the $\alpha$ subunit loop. We have determined the effects of ligands of the $\alpha$ and $\beta$ subunits on the rate of tryptic cleavage of the $\alpha$ subunit in the $\alpha_2\beta_2$ complex (Fig. 1A and B) and on the ratio of activity in the presence of $\alpha$-glycerol 3-phosphate to the activity in the absence of $\alpha$-glycerol 3-
Fig. 1. Effect of ligands on the time course of proteolysis of the tryptophan synthase αβ₂ complex as determined by sodium dodecyl sulfate gel electrophoresis (A), by densitometric analysis of the gels (B), and by determination of the relative activity (Activity + GP/Activity – GP) (C).

We find that L-serine alone has no effect on the rate of tryptic cleavage or of activation. In contrast, the α subunit ligand, α-glycerol 3-phosphate, decreases the rate of cleavage and of activation, as previously reported [11]. An important new observation is that addition of L-serine in combination with α-glycerol 3-phosphate almost completely prevents cleavage and activation. The rates of activation of the αβ₂ complex during proteolysis (Fig. 1C, curves 1–4) are similar to the rates of proteolysis (Fig. 1B, curves 1–4).

Table I shows the protective effects of ligands of the α and β subunits. L-Serine and low concentrations of L-tryptophan and D-tryptophan, which bind to the enzyme, give strong protection from cleavage and from activation in the presence of α-glycerol 3-phosphate but have much smaller effects in the absence of α-glycerol 3-phosphate. The nonsubstrate amino amino acids, L-alanine and D-alanine, have no effect in the presence or absence of α-glycerol 3-phosphate. In the presence of α-glycerol 3-phosphate, a high concentration of d-serine has a small effect whereas a low concentration has no effect.

4. DISCUSSION

The flexible loop in the α subunit plays important roles in ligand binding and in communicating the effects of ligand binding from the α subunit to the β subunit [11,14]. Our new finding that a ligand at the β site stabilizes the α subunit loop in the presence of a ligand of the α subunit is evidence that communication between the α subunit loop and the β site is reciprocal. Reciprocal communication between the α and β sites results from the transmission of ligand-induced conformational changes between the active sites [5–7]. Our finding that L-serine alone does not prevent loop cleavage shows that the effects of L-serine on proteolysis...
Table I

Effects of ligands of the α and β subunits on proteolytic cleavage of a flexible α subunit loop in the tryptophan synthase αβ2 complex

<table>
<thead>
<tr>
<th>Addition</th>
<th>α subunit ligand</th>
<th>Protection from cleavage (%)</th>
<th>Protection from activation (%)</th>
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<tr>
<td>None</td>
<td>GP</td>
<td>62</td>
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<tr>
<td>L-Serine</td>
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<tr>
<td>L-Alanine</td>
<td>50 mM</td>
<td>GP</td>
<td>57</td>
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</table>

* The αβ2 complex was treated with trypsin for 10 min in the presence or absence of the indicated β subunit ligand and α subunit ligand (50 mM d-α-glycerol 3-phosphate; GP) as described in Fig. 1.

% protection from cleavage = 100 x ([%α + ligands] - [%α - ligands])/(100 - [%α - ligands]), where [%α = % α subunit remaining after 10 min proteolysis as determined by densitometry. An example of the calculation for the first experiment with GP alone, % protection from cleavage = 100 x (69 - 26)/(100 - 26) = 58%.

% protection from activation = 100 x ((Tr(-L) - Tr(+L))/(Tr(-L) - UnTr)) is calculated from the relative activity (Activity + GP / Activity - GP) of enzyme treated in the absence of ligands (Tr(-L)), in the presence of ligand (Tr(+L)), or untreated (UnTr). An example of the calculation is given for the first experiment with GP alone: % protection from activation = 100 x (0.76 - 0.49)/(0.76 - 0.33) = 63%.

Differ from the effects of L-serine on subunit dissociation [14-17] and on heat denaturation (Ruvinov and Miles, unpublished results). L-Serine alone strongly protects the αβ2 complex from dissociation and from heat denaturation; the addition of L-serine and α-glycerol 3-phosphate in combination results in stronger protection. We conclude that our studies reveal a specific effect related to the loop in the liganded α subunit. The loop may undergo a conformational change upon binding α-glycerol 3-phosphate that is further stabilized by an L-serine-induced conformational change in the β subunit. This hypothesis is supported by our finding that ligands do not protect and αβ2 complex with a mutation in the loop (T183A) [17] but do prevent several other mutant αβ2 complexes (Ruvinov and Miles, unpublished results). We anticipate that future crystallographic studies of the αβ2 complex with ligands bound at both α and β sites will disclose changes in the α subunit loop that result from ligand-mediated subunit communication.

Acknowledgement: We thank Dr. Steven B. Zimmerman for generous assistance in the use of his densitometer system.

REFERENCES