Intergeneric Complementation of Anthranilate Synthase Subunits

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Partially purified subunits of anthranilate synthase were prepared from Bacillus subtilis and Pseudomonas aeruginosa. The large component from B. subtilis (I₈) complements well with the small component from P. aeruginosa (I₉) to reconstitute a glutamine-reactive anthranilate synthase. This interaction can be demonstrated with crude extracts from a B. subtilis trpX mutant and a P. aeruginosa trpA mutant. Complementation was also observed with the large component from P. aeruginosa (I₈) and the small subunit from B. subtilis (I₉). At saturation the heterologous complex I₈I₉ has 93% of the activity of the homologous complex I₈I₈, whereas the hybrid I₈I₉ is only 22% as active as the homologous complex I₈I₈.

Anthranilate synthase is the first enzyme in the tryptophan biosynthetic pathway. In all bacteria studied thus far, this enzyme is composed of two nonidentical subunits (H. Zalkin, Advan. Enzymol., in press). The large subunit, component I, catalyzes the formation of anthranilate by using ammonia as a substrate but cannot utilize glutamine as the amide donor. The small subunit, component II, has no anthranilate synthase activity by itself but complexes with the large subunit to give an aggregate that can use glutamine as a substrate. Thus, a test for complementation is established by mixing subunit preparations and assaying for the glutamine-reactive anthranilate synthase activity that occurs as a result of the formation of hybrid complexes. The anthranilate synthases from Bacillus subtilis (5) and Pseudomonas aeruginosa (9) are readily dissociable enzyme aggregates. This property distinguishes them from the anthranilate synthase complex of Serratia marcescens, Escherichia coli, Enterobacter aerogenes, and Salmonella typhimurium, all of which are tightly aggregated (H. Zalkin, Advan. Enzymol., in press). Intergeneric complementation has been demonstrated between the isolated subunits of E. coli, S. typhimurium, and E. aerogenes (2, 3), but no complementation could be demonstrated between components from P. aeruginosa or P. putida and S. typhimurium (2). Complementation was found to occur between several species of Pseudomonas (9), and two classes of anthranilate synthase (that is, readily dissociated and tightly aggregated) were found within this genus. Complementation within these classes was greater than that observed between the classes (9). Similarly, complementation could be demonstrated between species with a tightly aggregated enzyme (e.g., P. testosteroni) and S. typhimurium. Since B. subtilis has a readily dissociable enzyme, we examined the complementation between the enzyme subunits from this gram-positive microorganism and the subunits from the gram-negative rod P. aeruginosa.

MATERIALS AND METHODS

The characteristics of the B. subtilis mutants NP100, I-12, and I-15 have been described (5, 7). The isolation of partially purified subunits I₈ and I₉ from mutant NP100 has also been detailed (6). The P. aeruginosa mutants, 4FT-1 and trpAl, were obtained from Roy A. Jensen. The mutant 4FT-1 is a tryptophan excretor that has a genetically derepressed anthranilate synthase (1); mutant trpAl lacks a catalytically functional large component of anthranilate synthase (R. Jensen, personal communication) but retains a normal small subunit. The I₈I₉ complex from P. aeruginosa 4FT-1 was obtained by chromatography on diethylaminoethyl-cellulose. The fractions containing the I₈I₉ complex were pooled, concentrated, and applied to a Sephadex G-100 column (2.5 by 80 cm) equilibrated with 20 mM tris(hydroxymethyl)aminomethyl (Tris)-hydrochloride buffer, pH 7.0, containing 6 mM β-mercaptoethanol. The large component, I₉, was found by assaying column eluates for the ammonia-reactive anthranilate synthase activity. These fractions had no demonstrable activity with glutamine as a substrate. The small component, I₈,
which has no anthranilate synthase activity itself, was found by assaying for the glutamine-reactive anthranilate synthase activity in the presence of crude extract from mutant I-15.

All strains were grown in minimal salts glucose medium (4) except mutant trpAl which was grown in a chemostat in the presence of a growth-limiting concentration of L-tryptophan. Extracts of the B. subtilis mutants were prepared and lysed as described before (8) except that 0.5 M KCl was omitted from the buffer. P. aeruginosa extracts were prepared in the same buffer, but the cells were disrupted by 2 min of sonic treatment at 0°C. Glutamine-reactive anthranilate synthase activity was determined fluorometrically (excitation wavelength, 313 nm; emission wavelength, 393 nm, both uncorrected) in a 1-ml reaction mixture containing 60 μmol of Tris buffer (pH 7.5), 10 μmol of MgCl₂, 20 μmol of glutamine, and 400 nmol of chorismate. The reaction was started by the simultaneous addition of the appropriate anthranilate synthase subunit fraction. In some cases there was a 2- to 4-min lag before steady-state velocity was reached; therefore, all rates shown are final steady-state velocities.

RESULTS AND DISCUSSION

Neither B. subtilis mutants I-12 (lacks subunit I₈) nor I-15 (lacks subunit I₈) have activity in the in vitro assay for glutamine-reactive anthranilate synthase (7). When these two extracts are mixed, however, activity is reconstituted (Fig. 1). The P. aeruginosa mutant trpAl, which is analogous to the B. subtilis mutant I-12, lacks a catalytically functional large component, I₈, of the anthranilate synthase aggregate. A glutamine-reactive anthranilate synthase activity can be demonstrated upon the addition of crude extracts from mutants I-15 and trpAl (Fig. 1). This indicates that the small component II₈ can complement the large component I₈ even in the presence of the defective subunit II₈.

The data in Fig. 2 illustrate the complementation that is observed with partially purified subunit fractions from P. aeruginosa and B. subtilis. Increased concentrations of partially purified subunits II₈ and II₆ are added to a fixed level of components I₈ (Fig. 2A) and I₆ (Fig. 2B). In most cases the reassociation of the subunits is characterized by a hysteretic lag of 2 to 4 min (6) before steady-state velocities were reached. Therefore, the appropriate concentration of subunits were mixed together in the presence of 20 μmol of glutamine in a volume of 0.15 ml and allowed to stand at room temperature for 2 min. After this preincubation, 60 μmol of Tris buffer (pH 7.5), 10 μmol of MgCl₂, and 400 nmol of chorismate were added to give a final volume of 1 ml. Under these conditions the lag in velocity was minimized. Saturation of component I₈ was reached by using either component II₈ or II₆ (Fig. 2A). However, the activity of the I₈II₆ complex at saturation was only 22% of the activity of the homologous complex I₈II₈. Similarly, component I₆ was saturated with both II₈ and II₆ (Fig. 2B). In this case, however, the hybrid complex I₆II₈ had 93% of the activity of the homologous complex I₆II₆.

![Fig. 1. Complementation between crude extracts of B. subtilis and P. aeruginosa. The activity of the glutamine-reactive anthranilate synthase with 465 μg of protein from a crude extract of either mutant I-12 or trpAl is plotted as a function of protein concentration from a crude extract of mutant I-15.](http://jb.asm.org/)

![Fig. 2. Complementation with partially purified subunit fractions. A, Rate of the glutamine-reactive anthranilate synthase with 5 μg of protein containing partially purified component I₈ is plotted as a function of the quantity of protein containing partially purified components of II₈ or II₆. B, Rate of the glutamine-reactive anthranilate synthase with 1 μg of protein containing partially purified component I₈ is plotted as a function of the quantity of protein containing partially purified components II₈ or II₆.](http://jb.asm.org/)
The possibility of the small component serving as a glutaminase without actually forming an active hybrid enzyme complex was considered, but the following data are not consistent with this interpretation. (i) The small components $\Pi_0$ and $\Pi_F$ were preincubated with glutamine for 5 min at 37°C prior to the addition of components $I_P$ or $I_0$, respectively. If the small components were releasing ammonia via a glutaminase activity, then the velocity should be greater for the preincubated samples than for the zero time controls. There was, however, essentially no change in the velocity ($0 - 0.060$ nmol/min; $5 - 0.058$ nmol/min) as a result of preincubation. (ii) With $5 \mu$mol of NH$_4$Cl as a substrate instead of 20 $\mu$mol of glutamine the rate of anthranilate produced by components $I_0$ and $I_P$ was only 25% of the rate observed with glutamine as the substrate. Therefore, it seems unlikely that a concentration of NH$_4^+$ or NH$_3$ necessary to account for the observed enzyme rates with these assay conditions could be generated by a free-glutaminase activity during the time of assay.

Rather, these data indicate protein-protein interactions to be responsible for the glutamine-reactive anthranilate synthase activity that is found with the hybrid complexes. The qualitatively positive complementation between taxa as different as B. subtilis and P. aeruginosa suggests that this experimental system has much to offer in the consideration of evolutionary relationships.

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LITERATURE CITED