**β-Galactosidase α-complementation**

* A model of protein-protein interaction

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**Summary**

Studies on β-galactosidase α-complementation are reviewed. The isolation and structure of two β-galactosidase fragments that form an enzymically active complex are described. One of these is a cyanogen bromide peptide from whole β-galactosidase; the other is a dimeric protein from a lacZ deletion mutant of *Escherichia coli*. The mechanism most likely involves an initial binding of two cyanogen bromide peptides to the dimer, followed by formation of a tetramer, and finally a slow conformational change of the complex to a native-like enzyme. The overall reaction is essentially irreversible. A region of the polypeptide chain involved in dimer–dimer contact must be supplied by the cyanogen bromide peptide. α-Complemented enzyme contains overlapping sequences. Proteolytic experiments were carried out to determine the origin of the functionally important segment. The effect on α-complementation of amino acid substitutions at four positions in the polypeptide chain was investigated. The implications of these results for β-galactosidase structure and for proteins in general are discussed.

**Introduction**

Complementation, originally in the province of geneticists concerned with the definition of the gene and the analysis of gene function (cf 1), has become of more and more interest to biochemists studying polypeptide chain interaction and normal folding processes of proteins. To complement a protein is to restore its function by completing it, by supplying a correct or a missing polypeptide. Protein complementation involves non-covalent interaction of polypeptide chains and may occur between more than one different chain (intercistronic) or between fragments of a single chain (intracistronic).

α-Complementation in β-galactosidase is intracistronic. β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) of *Escherichia coli* is a tetrameric enzyme containing four identical chains each containing 1021 amino acids (2). Studies on α-complementation have helped in the determination of the primary structure, and are yielding some insights into higher order structure of β-galactosidase, as discussed in this paper.

**Early experiments**

α-Complementation in β-galactosidase was discovered in extracts of lacZ (coding for β-galactosidase) mutants of *E. coli* (3). It occurs in vivo, in partial diploids, and in vitro, with extracts of certain mutant strains. In the latter case, when extracts of two specific kinds of mutant strains were mixed and allowed to stand at room temperature for an hour or two, β-galactosidase enzyme activity, absent from each of the initial extracts, was found to be restored. One of these strains carried a mutation in the operator-proximal, or α part of the gene; the second had the α region intact but had a mutation elsewhere in the gene. Two complementing mutant
strains are illustrated in Fig. 1. *lacZM15* is a deletion mutant that maps in the α region. The defective β-galactosidase protein produced by this strain is the α-acceptor. *lacZU239* is a nonsense mutant whose termination codon maps near the center of the gene. The prematurely terminated polypeptide formed is the α-donor.

A very unusual finding was made by Morrison and Zipser who autoclaved extracts of many *lacZ* strains that contain α-donors and found that the soluble fraction contained α-donor activity (4). Of course, most proteins in the extract denature and precipitate in the autoclave, but these conditions also cause some covalent bond cleavage. When the soluble fraction was examined in a sucrose-urea gradient, considerable heterogeneity was seen, but the activity was found to be in a fraction corresponding to a size of about 7400 daltons.

The next advance in the study of α-complementation was the finding that a mixture of cyanogen bromide peptides obtained by cleavage of β-galactosidase had α-donor activity (5). The active fraction was in the molecular weight range of 8–11,000. It was present in digests of extracts of strains with intact α-regions, like *lacZU239*, but was, as expected, absent from *lacZM15*. Cyanogen bromide cleaves specifically at methionyl residues in a polypeptide chain. Assuming as seemed likely, that the activity resided in a single peptide, this meant that the α-donor activity could be studied in a specific, defined segment of the polypeptide chain. Furthermore, since the position of the *lacZM15* deletion was known from genetic mapping to be near the beginning of the gene, the α-donor peptide must be derived from the amino terminal part of the polypeptide chain.

**α-Complementation as an analytical tool**

Like assays for complete β-galactosidase, α-complementation can be used to measure α-donor or α-acceptor fragments of β-galactosidase. Their exact chemical nature need not be known. For one study comparing the rates of synthesis and degradation of different nonsense mutant fragments (6), α-donors were assayed in whole cells, by treatment of bacterial pellets with cyanogen bromide. After removal of reagents by evaporation, an extract containing excess α-acceptor was added, and complemented enzyme was measured in the usual way. It was found that rates of synthesis of various prematurely terminated chains were the same but that rates of degradation differed. In general, short chains were degraded very rapidly, while longer polypeptides were more stable.

In another set of experiments concerned with whether growing polypeptides begin to fold before completion of biosynthesis (7) the quantity of incomplete chains was measured by treatment of ribosomal fractions with cyanogen bromide. Evidence obtained with an immunological procedure indicated that β-galactosidase polypeptides begin to fold while still ribosome-bound.

α-Donor fragments can also be assayed after preliminary treatment in the autoclave. This procedure is perhaps more convenient for certain experiments but is less sensitive by a factor of 7- or 8-fold.

Assays by α-complementation have been used in many other studies including those of polarity in the Lactose Operon (4), and on the binding of fragments to affinity columns (8). The isolation and purification of a number of fragments of β-galactosidase have been monitored by α-complementation.

**Isolation of α-donor**

On cleavage with cyanogen bromide, β-galactosidase yields 24 cyanogen bromide peptides ranging in size from 2 to 119 amino acid residues (2). Isolation in pure form of the α-donor peptide was made considerably easier by the complementation test. The protein was carboxymethylated before cleavage. This did not affect complementation. After preliminary separation of large and small CNBr peptides on Sephadex G-25 in 30 percent acetic acid, the fraction containing large peptides was found to have the α-donor activity. This was chromatographed on a carboxymethyl cellulose column in ammonium acetate buffer, pH 5, containing 6 M urea, and purified further on Sephadex G-50. The α-donor peptide was obtained as a single compo-