β-GALACTOSIDASE: α-COMPLEMENTATION OF A DELETION MUTANT WITH CYANOGEN BROMIDE PEPTIDES *

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SUMMARY

Peptides produced by cleavage of aminoethyl β-galactosidase with cyanogen bromide complement extracts of M15, a mutant with a deletion in the α region of the z gene. Determinations by gel filtration and acrylamide gel electrophoresis in sodium dodecyl sulfate show that the active fraction is in the molecular weight range of 8-11,000. Peptides produced by cyanogen bromide cleavage of crude extracts of strains with intact α regions also complement with M15. In contrast, cyanogen bromide peptides from M15 and from an uninduced wild-type strain do not complement.

In vitro complementation can be defined as the restoration of a given enzyme activity by combining two protein fractions, each enzymatically inactive as a result of a mutational event. Complementation between β-galactosidase mutant strains of Escherichia coli has been studied by Perrin (1) and Ullmann and co-workers (2-4). One class of such reactions, α complementation, involves mutations in the operator-proximal part of the z gene (the structural gene for β-galactosidase, Fig. 1). This initial segment of the gene specifies the amino terminal of the β-galactosidase polypeptide chain (5,6). When an extract from a mutant having a deletion in the α segment (α acceptor) is mixed with an extract from a strain whose α region is intact (α donor), enzyme activity is restored.

These complementation reactions have been studied in crude extracts.

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However, an α donor fragment has been identified by Sephadex fractionation to be a polypeptide chain with a molecular weight of about 25,000 (3). Recently, Morrison and Zipser (7) have shown that a soluble polypeptide fraction, obtained by autoclaving crude extracts of wild-type strains containing β-galactosidase or by autoclaving extracts of certain mutant strains, has α donor activity. This fraction has a molecular weight of approximately 7400 estimated by sucrose-urea density gradient centrifugation. We were stimulated by this finding to test cyanogen bromide (BrCN) peptides derived from pure β-galactosidase as α donors. While these experiments were in progress complementation of missense mutants of the tryptophan synthetase A protein by BrCN peptides was reported by Jackson and Yanofsky (8).

The total mixture of peptides from β-galactosidase (9) which had been aminoethylated (10) and cleaved with BrCN (11) was found to serve as α donor for extracts of the deletion mutant M15. The complementation assay was carried out by the addition of a suspension of peptide mixture in 0.1 M NaH_2PO_4, 2×10^{-3} M $MgSO_4$, 2×10^{-4} M $MnSO_4$, 5×10^{-2} M mercaptoethanol, pH 7.0, to a crude extract of M15 in a final volume of 0.2 ml. After 2 hours of incubation at 28°, β-galactosidase enzyme activity was determined by the standard assay (12). This activity was found to be dependent on the amount of donor peptides as well as the amount of M15 extract. At saturating levels of donor peptides, an assay mixture containing 1 mg. of M15 extract protein produced about 1,000 units of β-galactosidase activity. This was a considerable restoration of activity, since an equivalent amount of extract of a fully induced wild-type strain would contain approximately 10,000 units. M15 has no enzyme activity by itself.

The BrCN peptides were also tested for complementation activity against two other mutants, W4680 and X90. W4680 is a mutant with a deletion at the middle portion of the z gene whereas X90 is an ochre mutant in which the site of mutation is at the extreme operator-distal end of the gene (Fig. 1). In contrast to the M15 experiments, when the BrCN peptides were added to extracts

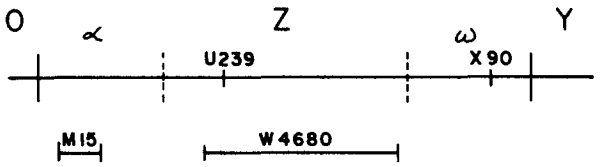

Fig. 1. Map of β-galactosidase mutant strains. Map positions and mutant strains according to Ullmann, Jacob and Monod (4).

of these two strains in the above system, no β-galactosidase activity was restored.

To find the peptide(s) containing the α donor activity, the BrCN peptide mixture was separated into soluble and insoluble fractions by extraction with 0.1 M pyridine-acetic acid buffer, pH 6.5. The insoluble fraction, which contained over 98% of the α donor activity when assayed under conditions in which the donor peptides were limiting, was applied in 30% acetic acid to a Sephadex G100 column equilibrated with the same solvent. The eluate was separated into Fractions I-V (Fig. 2) and Fraction III was found to contain 83% of the α donor activity. Further purification of the peptides in this fraction is now in progress.

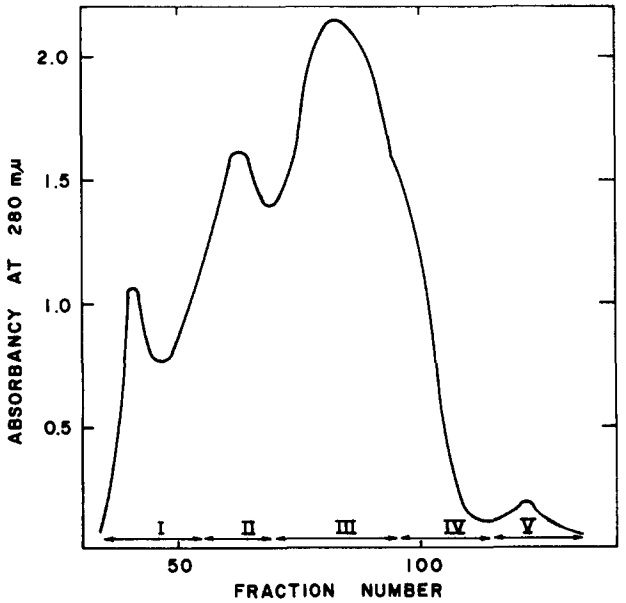

Fig. 2. Fractionation of the insoluble BrCN peptides on Sephadex G100 in 30% acetic acid.

To approximate the size of the active peptide(s), Fraction III, lysozyme, and insulin were fractionated on the same G100 column (Fig. 3). The elution volume of the α activity peak compared to that of the two standard proteins indicates that the active material has a molecular weight of about 11,500. The size of the active peptide(s) was also determined by electrophoresis on 12.5% acrylamide gel in 0.1% sodium dodecyl sulfate using hemoglobin, lysozyme, cytochrome c, insulin and a 42 residue peptide of known molecular weight (kindly supplied by Dr. E. L. Smith) as standards (13). After staining with Coomassie brilliant blue, two main bands were seen in positions corresponding to molecular weights of 8,000 and 11,000, respectively. These results together with the values determined by Sephadex fractionation indicate that the peptide(s) with α donor activity contains about 70 to 90 residues. This precludes the possibility that the peptides are actually undegraded β-galactosidase.

Crude extracts were also treated with BrCN to test whether these mixtures rather than the pure protein could serve as the source of α donor peptides.

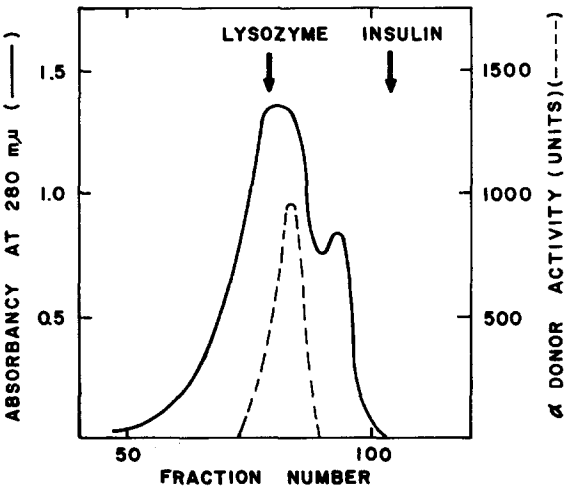

Fig. 3. Refractionation of Fraction III on Sephadex G100. Arrows refer to the positions of elution of the two standard proteins.

This technique could be useful as a quantitative measure of certain mutant polypeptide chains. Bacterial cells were broken by sonication, the particulate matter was removed by centrifugation at 40,000 g for 30 minutes and the

Table I

Complementation of M15 with BrCN-treated Extracts

Strain (α donor)	Enzyme Units
Wild-type (Hfr 3000) Induced	2,300
Wild-type Uninduced	< 4
M15	< 4
U239	420
X90	950

Activity is expressed as enzyme units per mg. of protein in donor extracts with an excess of acceptor protein.

clear supernatant solution was made 70% in formic acid. Cleavage by a quantity of BrCN equal to twice the weight of protein was allowed to proceed at room temperature for 24 hours. The solutions were diluted with water and lyophilized to dryness, the residues were suspended in buffer and were tested for α donor activity with M15 as acceptor (Table 1). A non-induced culture of a wild-type strain (Hfr 3000) has no α donor activity, nor does the peptide mixture produced by cleavage of M15 itself. On the other hand, α donor activity was found in BrCN-treated extracts of the induced wild-type strain, and of the nonsense mutant strains U239 and X90. Evidently, complementation can take place despite the presence of large numbers of nonspecific peptides.

These experiments indicate that the amino acid sequence missing in the polypeptide chain produced by the deletion mutant strain is supplied by the active peptide or peptides in Fraction III. It seems probable that the active donor is a single peptide. However, treatment of proteins with BrCN sometimes converts methionyl residues to homoserine with only partial cleavage of the polypeptide chain (14). Since the position of the deletion is known from genetic studies, the complementation experiment places the peptide(s) within

a certain area of the polypeptide chain. This is an important aid to the sequence determination of β-galactosidase in progress in this laboratory.

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