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β-GALACTOSIDASE ω-COMPLEMENTATION WITH A SMALL CYANOGEN BROMIDE PEPTIDE

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SUMMARY: Complementation has been found to occur between extracts of lacZX90, an operator distal nonsense mutant of E. coli, and CNBr24,the 32-residue carboxyl-terminal cyanogen bromide peptide from β-galactosidase. The degree of complementation is less than between X90 protein and a large carboxyl terminal fragment from lacZB9 but the times required are similar. Other peptides including the 10-residue carboxyl terminal tryptic peptide are ineffective in complementation.

INTRODUCTION:

Intracistronic complementation is the association between differently altered mutant proteins or protein fragments from the same cistron to give biologically functional protein. For β-galactosidase of Escherichia coli, omega(ω)-complementation has been defined as complementation that occurs between an acceptor protein comprising at least the amino terminal two-thirds of the polypeptide chain and a donor consisting of the carboxyl terminal third of the molecule (1, 2). It has been proposed that the ω-donor forms an intact globule or domain which then interacts with the ω-acceptor protein (3). Immunological experiments support this view because antibodies raised against ω-donors react well with native β-galactosidase (4).

We have asked whether ω-complementation might occur between an acceptor protein and a donor fragment smaller than one-third of the polypeptide chain. In this report we present experiments on the ability of peptides derived from the carboxyl terminal region of β-galactosidase to complement extracts of the nonsense mutant lacZX90 which maps near the end of the gene. Strain lacZX90 produces a prematurely terminated β-galactosidase lacking only a small segment at the carboxyl terminus. These results are compared to complementation between the large donor fragment from lacZB9, a polypeptide of about 40,000 daltons, and X90 protein.

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MATERIALS AND METHODS:

The bacterial strains 8058(F'pro,lacZX90/Δ(pro,lac),degT⁻) (5) and lacZB9 (1) were the sources of ω-acceptor and ω-donor, respectively. Cultures were grown on minimal media with 0.4% glycerol at 30°C. Cells from 6 liters of each culture were broken open by sonication and treated with 5% streptomycin sulfate. After removal of nucleic acids, ammonium sulfate was then added to the extracts and the 0-50% (X90) and 0-70% (B9) precipitable material was resuspended and dialysed against 0.1 M sodium phosphate buffer pH 7.2, containing 5 mM β-mercaptoethanol and 10% glycerol. The protein concentration of each solution was approximately 45 mg/ml.

Cyanogen bromide and tryptic peptides were purified from carboxymethyl β-galactosidase as previously described (6, 7). Conditions of ω-complementation were the same as those for α-complementation (8).

RESULTS:

Four peptides derived from whole β-galactosidase were tested for complementation with X90 protein. As can be seen in Fig. 1, only CNBr24, from residues 990-1021 of β-galactosidase, was effective as an ω-donor. CNBr23, residues 967-989, and the tryptic peptides, T79, residues 972-1011, and T80, residues 1012-1021, were completely inactive.

The time course of ω-complementation with CNBr24 and, for comparison, the protein from lacZB9 are shown in Fig. 2. Under the conditions of these experiments the time required for maximum complementation of X90 protein is similar, about 2 hours for CNBr24 and 1 hour for B9 protein.

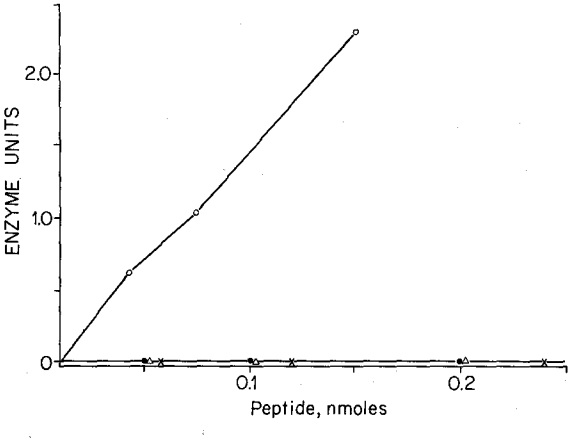

Fig. 1. Complementation between X90 protein and CNBr24 (o-o), CNBr23 (x-x), T79 (Δ-Δ), or T80 (•-•). The indicated levels of peptides were incubated for 3 hrs with 15 μl of X90 protein in a total volume of 200 μl and then assayed for β-galactosidase activity.

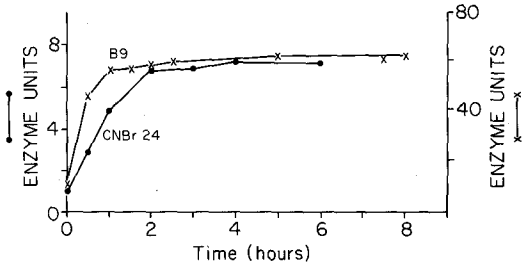

Fig. 2. Time course of complementation between X90 protein and CNBr24 (●—●) or B9 protein (x—x). 15 μl of X90 protein and 3 nmoles of CNBr24 or 25 μl of B9 protein were incubated in a total volume of 200 μl for various time intervals and then assayed for β-galactosidase activity.

It is possible to saturate X90 protein by increasing the concentration of either CNBr24 or B9 protein (Fig. 3). However, in order to achieve reasonable levels of β-galactosidase activity much more X90 was used during complementation with CNBr24 than with B9. These results and those in Fig. 2 indicate that the ω-donor activity of CNBr24 is more than 10-fold lower than that obtained with B9 protein.

DISCUSSION:

We have shown that CNBr24, the 32 residue carboxyl terminal cyanogen bromide peptide of β-galactosidase, can act as an ω-donor to complement X90 protein. The X90 protein produced in a strain containing a deg T⁻ mutation is indis-

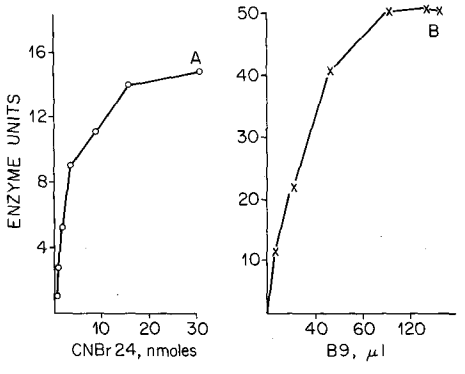

Fig. 3. Saturation curves of X90 protein with CNBr24 and B9 protein. 30 μl of X90 protein and indicated levels of CNBr24 (A) or 1 μl of X90 protein and various amounts of B9 protein (B) were incubated for 3 hrs in a total volume of 200 μl and then assayed for β-galactosidase activity.

tinguishable in size from that of wildtype on SDS polyacrylamide gels (9, and unpublished results, this laboratory). We estimate that the X90 molecule lacks no more than 20 of the 1021 amino acids of the whole polypeptide chain. The CNBr24 peptide of β-galactosidase therefore provides the missing amino acids. Interestingly T80, the 10 residue carboxyl terminal tryptic peptide of β-galactosidase does not complement X90 protein.

LacZB9 produces a protein of about 40,000 daltons. Our results indicate that the complete fragment is unnecessary for ω-complementation, though it is considerably more effective than CNBr24. There are several possible explanations for this. Extracts of strain 8058 contain X90 protein and in addition a degradation product of about two-thirds its size (9). Both would be expected to be active as ω-acceptors with B9 protein, but only the whole X90 protein could be complemented by CNBr24. Secondly, CNBr24 contains a carboxymethyl cysteine residue (from residue 1019 of carboxymethyl β-galactosidase) while B9 protein contains no carboxymethyl groups. Although carboxymethylation of native β-galactosidase has little or no effect on its enzyme activity (10), it is possible that the presence of a carboxymethyl group on CNBr24 may decrease its effectiveness as an ω-donor. Finally, B9 protein most likely supplies a preformed domain to the acceptor protein, while CNBr24 must interact and fold to form that domain. Hence it could be less efficient. Nevertheless, a study of ω-complementation between X90 protein and CNBr24 should be a useful model system for the study of protein-protein interaction.

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