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Position of the *lacZX90* Mutation and Hybridization Between Complete and Incomplete β-Galactosidase

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The position of the termination codon in *lacZX90* was determined by isolation of a *lac*$^+$ revertant. Lysine was found to replace tyrosine at position 1,012 of β-galactosidase, indicating that X90 protein lacked the carboxyl-terminal 10 residues. A heat- and urea-sensitive hybrid enzyme was formed in vivo when *supC*, which supplies tyrosine to the position in the polypeptide corresponding to the nonsense codon, was used to suppress *lacZX90*. This result shows that suppression that adds back the original amino acid may not lead to the production of the wild-type enzyme if the latter is multimeric, because incomplete chains can be incorporated into the oligomer.

β-D-Galactosidase of *Escherichia coli* (EC 3.2.1.23) is a tetrameric enzyme containing identical chains, each of 1,021 amino acid residues (9). Termination mutations in the gene specifying β-galactosidase, *lacZ*, have been used extensively in studies of this large enzyme (1). One in particular, ochre mutation *lacZX90*, which maps close to the operator-distal part of the gene (17), has been used to orient the protein to the gene (3, 8), to study folding of nascent chains on the ribosome (13), and to study protein degradation (5, 11, 14). The polypeptide chain specified by *lacZX90* (X90 protein) is devoid of β-galactosidase enzyme activity but can be complemented to form active enzyme by mixture with either a large fragment comprising the carboxyl-terminal third of β-galactosidase (21) or with a cyanogen bromide peptide, CNBr24, derived from the last 32 amino acid residues of the whole protein (22).

To map the *X90* mutation, a spontaneous revertant of strain 8058 [*F' lacZX90*(Oc)/Δ(*pro-lac*) *degT*] (14) was isolated by screening for *lac*$^+$ colonies on MacConkey plates. The X90 revertant strain was used as a source of β-galactosidase rather than a suppressed strain because the efficiency of suppression of ochre mutations is less than 5%. β-Galactosidase was purified as previously described (2). The purified protein had a specific activity of about 380,000 U/mg as compared to 450,000 U/mg for the wild-type enzyme and was at least 90% pure as estimated by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate. Electrophoresis in the absence of denaturant indicated that β-galactosidase from the X90 revertant strain, like the wild-type, was a tetramer. Higher multimeric forms were also present.

The protein was carboxymethylated and cleaved with cyanogen bromide. Since the size of the X90 polypeptide chain as estimated from sodium dodecyl sulfate gels is nearly the same as that of the wild-type chain, it was expected that the position of the mutation would be within the terminal 10 or 15 residues. Therefore, CNBr24 from the revertant protein was isolated. The isolation was performed in the same way as described for the wild type (6). The sequencer analysis of the peptide is shown in Table 1. The only difference from the wild type (7) was found to be the lysine residue at step 23, replacing tyrosine. This corresponds to position 1,012 of the β-galactosidase chain.

The placement of the *X90* mutation agrees with the DNA sequence known for the operator-distal part of *lacZ* (4), assuming that the nonsense mutation and its reversion involve single base changes. It can be deduced from the DNA sequence and the genetic code that the original tyrosine triplet (TAC) mutated to the ochre triplet (TAA) and is substituted in the *lacZX90* revertant by the lysine triplet (AAA) at the codon specifying residue 1,012.

lacZX90 was suppressed with *supB*, *supC*, and *supG*, which insert glutamine, tyrosine, and lysine, respectively, at the site of the nonsense codon (20). Suppressed strains were obtained by crossing strain 3000X90 [Hfr *lacZX90*(Oc) *thi*] with XA10B, XA10C, and XA105 suppressor strains [F$^-$ Δ(*lac-pro*) *ara argE nalA rif thi metB sup*)] (17) and selecting *pro*$^+$ clones resistant to nalidixic acid (50 μg/ml). The properties of enzyme from suppressed strains, the lysine revertant, and the wild type were compared.

Extracts of each strain were prepared, and the

TABLE 1. *Sequence analysis of the CNBr24 peptide*[a]

Step	Amino acid (wild type)	Amino acid (mutant)	Method of identification	Yield (nmol)
1	Gly	+	H	
2	Ile	+	H	400
3	Gly	+	H	
4	Gly	+	H, T	
5	Asp	+	H, T	
6	Asp	+	H, T	
7	Ser	+	H, T	
8	Trp	+	H, T	
9	Ser	+	H, T	
10	Pro	+	H, T	250
11	Ser	+	H, T	
12	Val	+	H, T	200
13	Ser	+	H, T	
14	Ala	+	H, T	100
15	Glu	+	H, T	140
16	Phe	+	H, T	100
17	Gln	+	H, T	
18	Leu	+	H, T	80
19	Ser	+	H, T	
20	Ala	+	H, T	60
21	Gly	+	H, T	60
22	Arg	+	AA	
23	Tyr	Lysine	H, AA	10
24	His	+	AA	
25	Tyr	+	H	
26	Gln	+	H	
27	Leu	+	H	40
28	Val	+	H	20
29	Trp	+	H	
30	Cys	+	H	
31	Gln	+	H	
32	Lys	+	H	

[a] Automated sequencing was done with 0.1 M Quadrol in a Beckman 890C sequencer. +, Identification of the residue. Methods of identification were high-pressure liquid chromatography (H), thin-layer chromatography (T), and amino acid regeneration (AA).

β-galactosidases were tested for heat stability. We were surprised to find that suppression of the X90 strain with *supC* (tyrosine) yielded an enzyme which was more heat sensitive at 50°C than was the wild type (Fig. 1A), because we had expected that wild-type enzyme would be formed. β-Galactosidase enzymes from *supB*- and *supG*-suppressed strains were also unstable and, in the latter case, much more unstable than the *lacZX90*$^{Rev}_{Lys}$ enzyme. The biphasic activity decay for the heat-unstable mutants at 50°C (Fig. 1A) suggested the presence of more than one enzyme species. At 57°C, *lacZX90*$^{Rev}_{Lys}$ β-galactosidase is also unstable but its activity loss is exponential, in contrast to *lacZX90-supC* enzyme (Fig. 1B).

Further evidence for a difference in structure between wild-type and X90 *supC* enzymes was obtained by testing stability in urea. Wild-type enzyme does not start losing activity until a concentration of 6 M urea is reached, whereas the mutant enzyme loses activity at 3 M urea (Fig. 2).

The most likely explanation for the heat and urea sensitivity of β-galactosidase from the suppressed strains is that, in the cell, hybrid oligomers are formed which contain complete and incomplete polypeptide chains. To test this possibility, wild-type β-galactosidase was dissociated in 8 M urea, mixed with X90 protein, and renatured by dialysis. The product proved to be heat unstable at 50°C, in contrast to a control sample renatured in the absence of X90 protein (Fig. 3). This in vitro experiment, therefore, mirrors the situation that occurs in whole cells. Hybrid β-galactosidase tetramers are known to

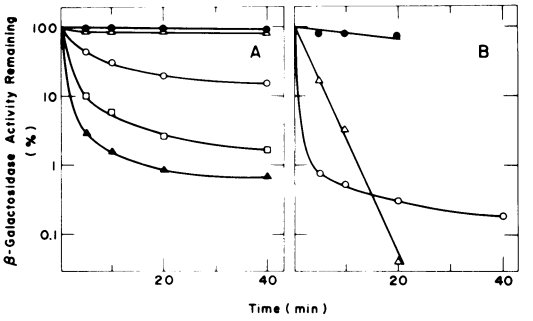

FIG. 1. *Heat stability of β-galactosidase. Crude extracts from the indicated strains were prepared in 0.1 M sodium phosphate buffer (pH 7.2). The protein concentration of each extract was 1.5 mg/ml. After incubation at 50°C (A) or 57°C (B), samples were assayed for β-galactosidase activity. Wild type, ●; X90*$^{Rev}_{Lys}$*, Δ; X90 supC (Tyr), ○; X90 supB (Gln), □; X90 supG (Lys), ▲.*

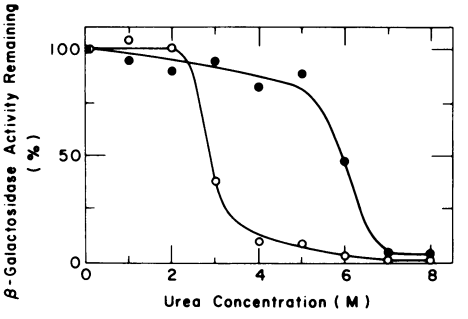

FIG. 2. *Urea stability of β-galactosidase. Wild-type β-galactosidase and the X90 supC enzyme were incubated for 2 h at room temperature with the indicated urea concentrations, and samples were diluted into buffer for β-galactosidase assays. Wild type, ●; X90 supC (Tyr), ○.*

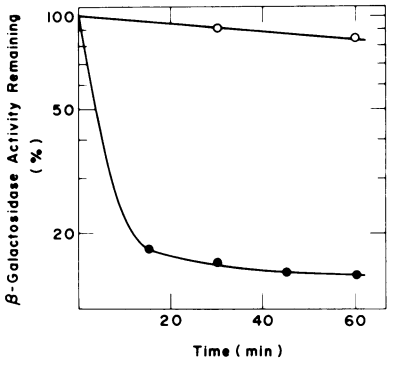

FIG. 3. *Heat stability of β-galactosidase renatured in the presence and absence of X90 proteins. Crude extracts of strain 3300 (wild type) containing 3,500 U of β-galactosidase activity in 0.5 mg of protein were mixed with an extract of a noninduced strain (HFr 3000)* (○) *and an extract of an induced X90 strain (8058)* (●) *to a final concentration of 5 mg of protein per ml and 8 M urea. After 2 h, the solutions were dialyzed against 0.1 M sodium phosphate-10^{-3} M magnesium sulfate-2 × 10^{-4} M manganese sulfate-0.1 M mercaptoethanol (pH 7.0). The recoveries of activity were 70 and 55%, respectively. Solutions were incubated at 50°C, and samples were assayed for activity.*

be formed both in vivo and in vitro from missense mutants (12, 15, 16, 19). To our knowledge, this is the first report of a hybrid oligomeric enzyme formed by suppression and containing complete and incomplete chains. No doubt such hybrids can also be formed in other oligomeric enzymes, providing, as is the case here, that the incomplete chain contains most of the sequence of the full polypeptide. This phenomenon is analogous to the effect obtained with i^{-D} mutants which negatively complement and, in some cases, are due to hybrid formation with restart fragments of the repressor (10).

We have reported earlier that the X90 protein is a monomer under normal conditions of ionic strength (3, 8). Therefore, residues missing from this chain must be important for monomer-monomer interaction. Clearly, other parts of the polypeptide must also take part in intersubunit binding or hybrid enzymes could not be formed.

X90 protein can complement with CNBr24, the terminal 32-residue peptide, but not with T80, the terminal 10-residue tryptic peptide derived from residues 1,012 through 1,021 (22). Therefore, the proper conditions for complementation are not met simply by supplying the acceptor (X90 protein) with the exact missing part of its sequence. It is evident that the terminal portion of the β-galactosidase polypeptide is critical for proper assembly of the molecule.

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