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In Vivo Complementation Between Wild-Type and Mutant β-Galactosidase in *Escherichia coli*

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A comparison of the specific activity of wild-type β-galactosidase synthesized in a *lacZ*$^-$/*lacZ*$^+$ heterogenote has shown that there is 60% more activity in the heterogenote's enzyme than can be accounted for by wild-type subunits alone. It is concluded that wild type β-galactosidase subunits can complement mutant subunits.

β-Galactosidase from *Escherichia coli* consists of four identical subunits (10) and possesses four active sites, one per subunit (11). The structural gene for β-galactosidase (EC 3.2.1.23) is the *Z* gene. It has been shown that heterogenotes with different *Z*$^-$ point mutations may form active β-galactosidase by complementation (4, 7). Crick and Orgel (1) suggested that certain mutations can cause local misfolding in a subunit and that this may be corrected by interaction with an adjacent region of another subunit which has correct folding. Complementation is thus interpreted as reflecting interactions between differently altered subunits. Most studies have been concerned with this sort of complementation, whereas complementation between wild-type and mutant subunits has been studied very little. The purpose of this study was to determine whether or not wild-type subunits are capable of complementing mutant subunits in vivo.

I have compared the specific activity of wild-type β-galactosidase synthesized in a haploid *Z*$^+$ strain with that of enzyme synthesized in a F′ *lacZ*$^-$/*lacZ*$^+$ heterogenote. The *Z*$^-$ allele chosen was mutation S30, a point mutation which leads to the production of β-galactosidase that enters into tetramers which sediment at 16*s* (as do wild-type tetramers), but which are virtually inactive in the absence of anti-β-galactosidase antibody (8). In my hands, extracts of fully induced strain S30 possessed less than 0.2% of wild-type levels of β-galactosidase activity. Similarly, Rotman and Celada (8) reported that the activity of this mutant enzyme is increased 550-fold by the addition of antibody.

In the absence of complementation between mutant and wild-type subunits, the specific activity (in units per milligram of β-galactosidase *monomer*) of enzyme synthesized in the *lacZ*$^-$/*lacZ*$^+$ heterogenote would be directly proportional to the fraction of wild-type subunits present. If P is the fraction of wild-type subunits in the heterogenote, complementation will yield enzyme with a specific activity *greater* than P times the wild-type specific activity.

All strains are *E. coli* K-12. *BH-1* is F$^-$ *lacZ*$^+$ *proC*$^-$ *met*$^-$. *BH-5* was prepared by introducing into *BH-1* an F′ episome carrying the *lac-Z*$^-$ mutation U118, an ochre mutation very close to the operator proximal end of the *Z* gene (6). Since the episome in *BH-5* synthesizes only a tiny N-terminal fragment of β-galactosidase monomer (which is not detected in our assay for monomer protein), the use of this strain serves as a control in measuring the effect of an F′ episome on the amount and specific activity of β-galactosidase. *BH-7* was prepared by introducing into *BH-1* an F′ episome carrying the *Z*$^-$ mutation S30 previously described.

The method for determining the amount of β-galactosidase monomer protein (both active and inactive) in an extract of *E. coli* has been previously described (3). The same method was used in this study except that the cells were labeled for 10 min with ^{3}H-leucine (2,000 mCi/mmol, 6.7 nmol/ml). Cells were induced with 2×10^{-4} M isopropyl-β-D-thiogalactopyranoside. Total protein synthesized during the labeling period was measured as total trichloroacetic acid-precipitable counts.

β-Galactosidase activity was determined from the rate of hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside in 0.15 M sodium phosphate buffer, pH 7.0. A unit is defined as the release of 1 nanomole of *o*-nitrophenol per min at 28 C.

TABLE 1. *β-Galactosidase in strains BH-1, BH-5, and BH-7*[a]

Strain	lac genotype	No. of experiments	Percent protein that is β-galactosidase	Specific activity of β-galactosidase (units/mg)
BH-1	Z^+ haploid	4	3.8	2.57×10^5
BH-5	$F' Z^{-U118}/Z^+$	2	3.25	2.51×10^5
BH1 and 5	(combined above)	6	3.62 ± 1.0	$2.53 \pm 0.14 \times 10^5$
BH-7	$F' Z^{-S30}/Z^+$	4	10.15 ± 1.7	$1.36 \pm 0.08 \times 10^5$

[a] Line 3 represents the combined data of lines 1 and 2. The data in lines 3 and 4 are presented as means ± the 95% confidence interval. Specific activity of β-galactosidase is in units per milligram of β-gactosidase *monomer*.

To estimate P, the fraction of wild-type subunits present in the heterogenote *BH-7*, I measured the fraction of total protein synthesized during ^{3}H-leucine labeling, which is β-galactosidase monomer. Table 1 shows the results of these measurements. Since the haploid Z^+ strain *BH-1* and the heterogenote control *BH-5* do not differ significantly in the percentage of total protein which is β-galactosidase, the mere presence of a F′ episome does not alter the amount of β-galactosidase monomer synthesized in a cell. Line four (Table 1) shows the results of four experiments with strain *BH-7*, the heterogenote which synthesizes both mutant and wild-type subunits. The ratio of the amount of β-galactosidase monomer in *BH-7* to that in *BH-1* and *BH-5* is 2.99 ± 0.15 (95% confidence interval). The increased amount of β-galactosidase monomer in the heterogenoate *BH-7* must be attributable to the episomally derived S30 subunits. This ratio gives an estimate of two episomally derived subunits per chromosomally derived subunit, a figure that agrees well with the estimate of two episomes per chromosome obtained by Frame and Bishop (2). Thus, in the heterogenote only one third of the subunits are wild type, and P = 0.33.

Table 1 also shows the specific activity of β-galactosidase synthesized in these strains. A comparison of the specific activities in strains *BH-1* and *BH-5* shows that the presence of an F′ episome does not itself alter the specific activity of the β-galactosidase. Line three shows the combined data of lines one and two. The specific activity of wild-type β-galactosidase was $2.53 \pm 0.14 \times 10^5$ units/mg. In the absence of complementation, the expected specific activity of enzyme in strain *BH-7* is 0.85×10^5 units/mg ($0.33 \times 2.53 \times 10^5$ units/mg). The observed specific activity of BH-7 enzyme was $1.36 \pm 0.08 \times 10^5$ units/mg. The observation that there is 60% more activity than can be accounted for by wild-type subunits is convincing evidence for complementation between mutant and wild-type β-galactosidase subunits. It should be noted that such complementation does not occur when extracts of induced S30 cells and wild-type cells are mixed (unpublished data).

Melchers and Messer (5) concluded, on the basis of in vitro renaturation experiments, that wild-type subunits do not complement mutant subunits. The contradiction between my results in vivo and their results in vitro may be attributable to imperfect associations between subunits assembled in vitro, or it may simply reflect a difference in the mutant alleles studied. On the other hand, my results agree with the findings of Schwartz (9), who reported that, in maize hybrid alcohol dehydrogenase composed of a wild-type and a temperature-sensitive subunit is as heat-stable as fully wild-type enzyme.

It is concluded that in vivo wild-type β-galactosidase subunits can complement mutant S30 subunits.

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