

Involvement of Sulfhydryl Groups in the β -Galactosidase of *Streptococcus lactis*

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Sulfhydryl oxidants and stabilizers caused changes demonstrating the sulfhydryl content of β -galactosidase for *Streptococcus lactis* 7962. Ammonium sulfate (0.85 M) rendered the enzyme insensitive to the oxidants. Titrations revealed 11.5 moles of sulfhydryl per mole of enzyme.

Among the lactic streptococci, only one strain (*Streptococcus lactis* 7962) has been shown to possess β -galactosidase (EC 3.2.1.23 β -galactose galactohydrolase). Previous reports (5, 6) described some of the properties of the β -galactosidase isolated from *S. lactis* 7962. The rest of the lactic streptococci utilize lactose through a phosphorolytic cleavage (7) which is thought to be similar to that described for *Staphylococcus aureus* (4).

Comparative studies of β -galactosidase from various sources have revealed enzymes with a variety of properties. In one such report, Erickson and Steers (3) compared β -galactosidase of *S. lactis* 7962 with that of several other bacteria. They suggested that the enzyme from this streptococcus contained sulfhydryl groups and that its instability was due to the oxidation of these groups. The present communication describes the sulfhydryl nature of the β -galactosidase from *S. lactis* 7962 and the effect of ammonium sulfate on the loss of enzymatic activity due to sulfhydryl oxidation.

The effect of the sulfhydryl oxidant *p*-chloromercuribenzoate (*p*-CMB) on the activity of β -galactosidase from *S. lactis* 7962 was tested; Fig. 1 shows the inactivation brought about when 0.02 mg/ml was added to a partially purified (40-fold) enzyme preparation. These data indicate that sulfhydryl groups may be involved at or near the active center on the enzyme. A previous report (5) described the stabilizing effect of ammonium sulfate on the activity of β -galactosidase from *S. lactis*. This effect as well as the protection from *p*-CMB inactivation afforded by 0.85 M ammonium sulfate can also be seen in Fig. 1. This protective effect is thought to be due to modifications in the enzyme in which the —SH groups become inaccessible to or incapable

of reacting with the *p*-CMB. Protection was also demonstrated when ethylenediaminetetraacetic acid (EDTA) was added, indicating that a metal ion may be involved in the structure of the enzyme; addition of 0.5 μ g of EDTA per ml stabilized the partially pure enzyme at 75% activity when stored at 5 C, whereas the untreated enzyme lost activity at about 1% per hr. Both the metal and the —SH groups may be internal structures, and the salt changes the molecular

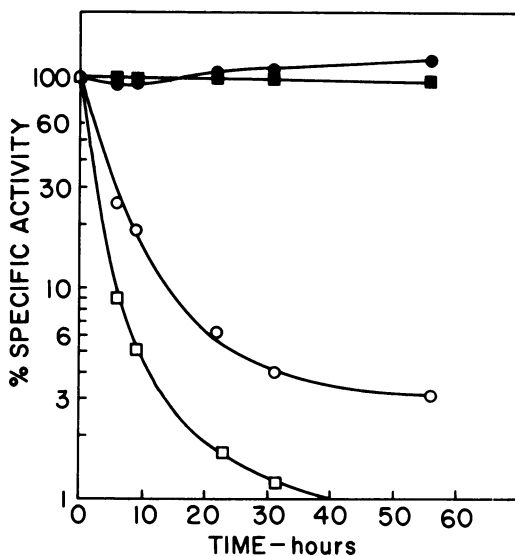


FIG. 1. Effect of *p*-CMB on specific activity of ammonium sulfate-stabilized and nonstabilized β -galactosidase from *Streptococcus lactis* 7962 at 5 C. The nonstabilized enzyme was in 0.1 M sodium phosphate buffer at pH 7.0, whereas the salt-stabilized enzyme was in the same buffer plus 0.85 M ammonium sulfate. Symbols: ●, salt-stabilized enzyme alone; ■, salt-stabilized enzyme plus 0.02 M *p*-CMB; ○, enzyme in buffer alone; □, enzyme in buffer plus 0.02 M *p*-CMB.

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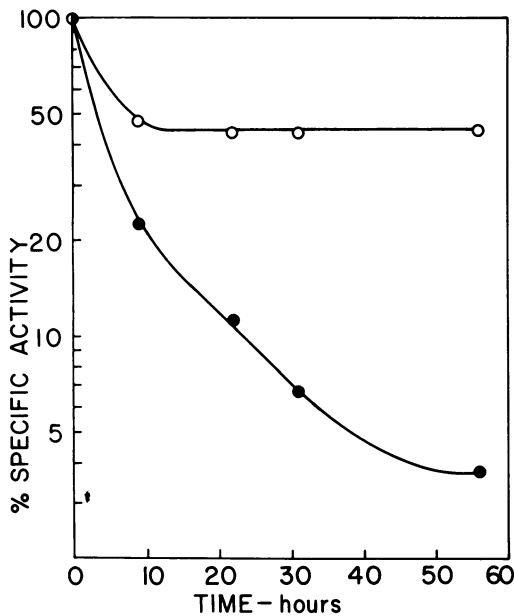


FIG. 2. Effect of addition of Cleland's reagent (0.02 mg/ml) to the β -galactosidase of *Streptococcus lactis* 7962 at 5 C. Symbols: \circ , Cleland's reagent added to enzyme in buffer; \bullet , enzyme in 0.1 M sodium phosphate buffer at pH 7.0.

conformation, making them unavailable to the environment. Another possibility is that the groups are located near or on the surface of the monomeric form of the enzyme, described elsewhere (5, 6), and that they are enveloped during dimerization which is promoted by ammonium sulfate.

The sulfhydryl stabilizer, Cleland's reagent, was added to partially purified enzyme solutions in buffer. The stabilization that resulted may be seen in Fig. 2. The effect of the stabilizer concentration appeared to be negligible over the range studied. Identical results were observed by using concentrations of Cleland's reagent, ranging from 0.25 to 5.0 mg/ml. These data also suggested the involvement of —SH groups in the enzyme structure, since a stabilization of 50% resulted. Other workers (3) have shown even greater stabilization with 2-mercaptoethanol.

Sulfhydryl titrations were carried out on enzyme that eluted as a homogenous peak of protein after gel filtration. A multiple-channel recording spectrophotometer was employed, and the method of Boyer (1) was followed by using $3\times$ purified *p*-CMB (8). There were 23 μ moles of sulfhydryl per mg of protein. These data were recalculated to express the number of —SH groups per molecule by using the previously reported (6) molecular weight of the monomer, 5×10^5 daltons. The calculated value was 11.5 moles of —SH per mole of monomer enzyme.

These results indicate the involvement of sulfhydryl groups in an essential portion of the β -galactosidase of *S. lactis*. In addition, this unstable enzyme can be rendered stable by the reduction of sulfhydryl groups or through conformational changes brought about by ammonium sulfate.

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