INACTIVATION AND DISSOCIATION OF β-GALACTOSIDASE BY AMINES

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SUMMARY

In addition to the inactivation and partial dissociation of β-galactosidase by mercaptoethanol, mercaptoethylamine or dithiothreitol, we found that ethanolamine or ethylenediamine rapidly inactivates the enzyme and causes quantitative dissociation of the 16S tetramer to a single 6S component. These results suggest that the thiol function of mercaptoethanol or mercaptoethylamine may be acting primarily as an electron donor in chelation rather than as a disulfide reducing agent.

INTRODUCTION

We have observed the inactivation and partial dissociation of β-galactosidase isolated from *Escherichia coli* K12 during relatively short incubation periods in the presence of 0.03M mercaptoethanol or 5×10^{-3}M dithiothreitol (1, 2). Inactivation with no reported dissociation in the presence of thiols had been observed by Reithel et al. (3) with the enzyme isolated from the ML308 strain of *E. coli*. We noted further (1, 2) that the enzyme was completely protected from inactivation or dissociation by magnesium or manganous ions. In addition, we observed (2) that the enzymatically inactive 8-9S dimers derived by thiol treatment from the active 16S tetramer could be reassociated into a catalytically inactive 16S aggregate on addition of divalent ions. While the mechanism of action of thiols is generally thought to be by reduction of some protein disulfide bonds which are essential for catalytic activity or subunit association, Craven et al. (4) reported that few if any of

the 76 residues of half-cystine in a mole of the enzyme are involved in disulfide bridge formation. Thus, in this case thiols did not seem to be acting by their usual mechanism. This led us to examine more extensively the properties of the functional groups of mercaptoethanol in order to determine the mechanism of inactivation and dissociation.

MATERIALS AND METHODS

β-Galactosidase was purified from *Escherichia coli* K12 strain 3300 by a modification of the procedure described by Craven et al. (4). The enzyme gave a single band on polyacrylamide disc gel electrophoresis and a monodisperse, homogeneous 16S boundary in the analytical ultracentrifuge.

The decay of catalytic activity was followed at 37° in 0.02M phosphate buffer, pH 7.5, by assaying aliquots at 30 minute intervals for residual activity with o-nitrophenyl-β-D-galactopyranoside (ONPG). The reaction was stopped by addition of 1M sodium carbonate and the absorbancy was read at 420 nm. The state of aggregation of the subunits was determined in the Spinco Model E analytical ultracentrifuge equipped with ultraviolet absorption optics, the electronic speed control and the photoelectric scanning system.

All reagents were purchased from Aldrich Chemical Company.

RESULTS

Rate of Inactivation by Thiols and Amines.- We previously observed that β-galactosidase is readily inactivated by 0.03M mercaptoethanol or 0.005M dithiothreitol in phosphate buffer, pH 7.5, at 37° (1, 2). If the inactivation depends upon the reducing properties of the thiol, we would anticipate that other monothiols should inactivate the enzyme at about the same

rate as found for mercaptoethanol. The decay of β-galactosidase activity in the presence of 0.01M mercaptoethylamine is compared in Figure 1 with that produced by an equimolar solution of mercaptoethanol. The more rapid rate of inactivation observed with mercaptoethylamine, coupled with the reported lack of cystine residues (4), led us to wonder if the thiol function is required at all for inactivation and/or dissociation of the enzyme. That inactivation will also occur when the thiol function is replaced with an amino group is shown by the curves for ethanolamine and ethylenediamine in Figure 1. It is clear that the amines inactivate the enzyme more rapidly than their sulfur analogues although the shape

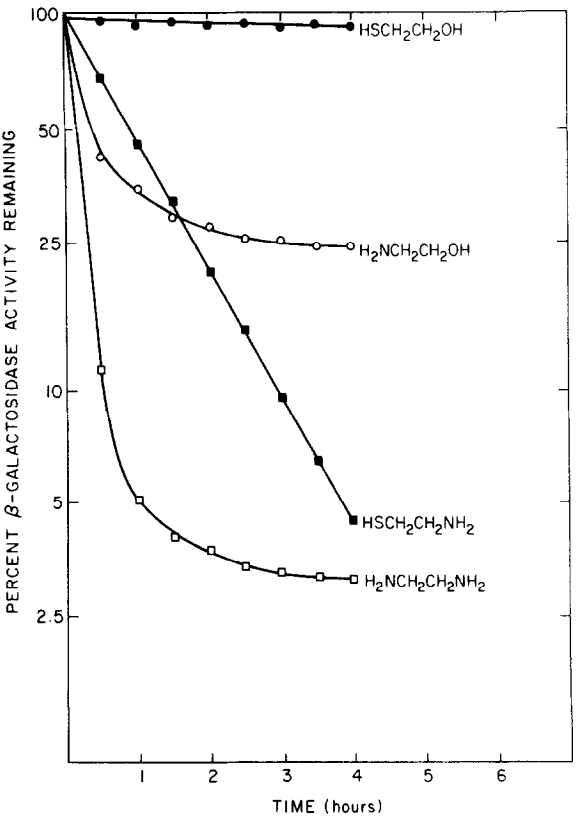

Figure 1. Rates of inactivation of β-galactosidase at 37° in the presence of 0.01M solutions of mercaptoethanol, mercaptoethylamine, ethanolamine and ethylenediamine.

of their decay curves is more complex than the simple first-order decay kinetics observed with the thiols.

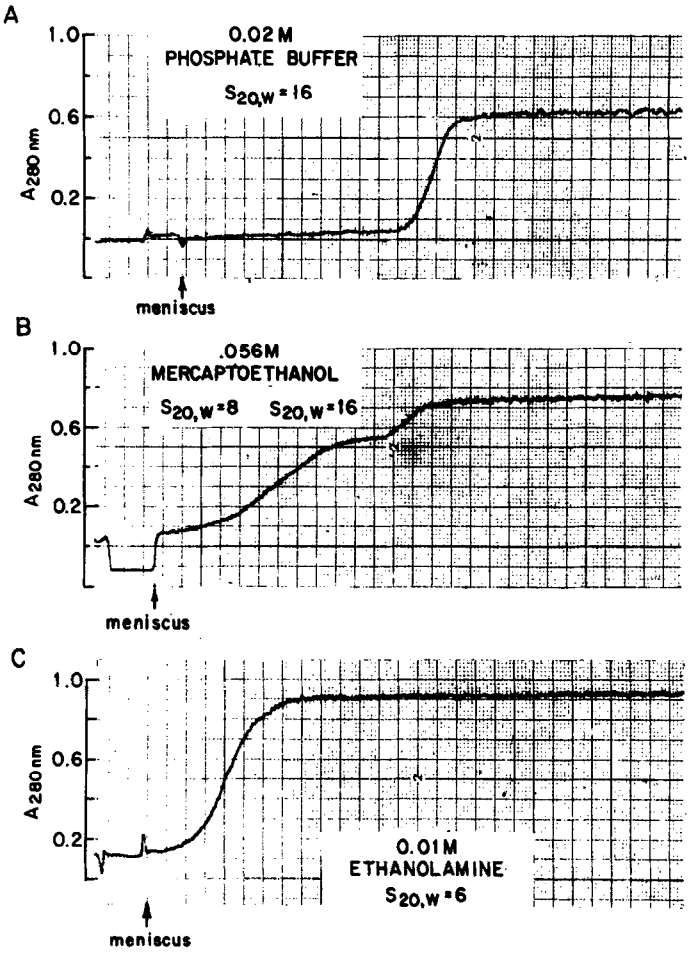

Figure 2. Sedimentation velocity patterns of A) native β-galactosidase (0.4 mg/ml) in 0.02M phosphate buffer, pH 7.5 (This pattern is unchanged after 15 hrs. in buffer at 37° (1, 2).); B) same concentration of protein after 15 hours incubation at 37° in 0.056M mercaptoethanol and c) under the same conditions as in B) except in the presence of 0.01M ethanolamine. All traces were made in a multicell run at 60,000 rpm, 25 minutes after acceleration of the rotor was begun.

Dissociation by Amines.- We had previously demonstrated (2) that nearly 85% of the 16S tetramer of β-galactosidase is dissociated by 0.056M mercaptoethanol into the 8S dimer. In

the presence of only 0.01M ethanolamine or ethylenediamine, the 16S tetramer is completely dissociated into a 6S component as shown in Figure 2. This Figure compares the sedimentation velocity patterns obtained from the native enzyme, the enzyme in 0.056M mercaptoethanol and in 0.01M ethanolamine.

DISCUSSION

The results presented here indicate that the thiol functions of mercaptoethanol or mercaptoethylamine are not specifically required for inactivation and/or dissociation. In fact, the corresponding amino compounds-ethanolamine or ethylenediamine- quantitatively dissociate the 16S tetramer into a homogeneous 6S component. Since the enzyme lacks disulfide bridges (4), thiols cannot act in their usual manner. Although the kinetics of inactivation by thiols and amines differ, it is reasonable to assume then that all of these compounds are functioning in a similar fashion, as chelators, through the electron donor properties of their hetero-atoms. In fact, the more effective inactivation and dissociation of the enzyme obtained with mercaptoethylamine as compared with mercaptoethanol is consistent with the greater electron donor properties of the amine nitrogen compared to the alcohol oxygen.

These data tentatively suggest that dimeric subunits of β-galactosidase are associated to the active tetramer through a divalent ion bridge. Further studies are being carried out to investigate this possibility more thoroughly.

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