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On the Reaction between *p*-Chloromercuribenzoate and Rhodanese

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Spectrophotometric measurements show that *p*-chloromercuribenzoate reacts slowly with crystalline rhodanese and a simultaneous inhibition of the enzyme activity is observed. In the presence of a detergent (dodecyl sulfate) a very rapid reaction is obtained, which allows the number of sulfhydryl groups in the enzyme to be determined. Both with PCMB and by amperometric titration with silver ions a value of 4 sulfhydryl groups per molecule rhodanese was obtained.

The enzyme rhodanese is inhibited by certain sulfhydryl reagents[1,2] and the presence of sulfhydryl groups in the crystalline enzyme has been demonstrated[3] by the nitroprusside reaction. However, as the inhibition experiments so far published have been carried out with dilute solutions of impure rhodanese and inhibitions have been obtained only at fairly high concentrations of inhibitor, the role of sulfhydryl groups in the enzymic reaction is very uncertain. One of the sulfhydryl reagents found to inhibit rhodanese, *p*-chloromercuribenzoate, has the advantage that its reaction with sulfhydryl groups can easily be followed spectrophotometrically. The present communication is concerned with the reaction between this compound and crystalline rhodanese.

MATERIALS AND METHODS

Crystalline rhodanese was prepared from beef liver as previously[3] described. A molecular weight of 37 100[3] has been used in the calculations. *p*-chloromercuribenzoic acid (PCMB) was a commercial product, which was dissolved in sodium hydroxide and then precipitated with hydrochloric acid. This purification step was repeated and the product then washed with distilled water and dried *in vacuo*. Other compounds were commercial products which were used without further purification. Sulfhydryl groups were determined spectrophotometrically with PCMB according to Boyer[4] and by amperometric titration with silver ions in tris buffer according to Benesch *et al.*[5]. Rhodanese activity was determined by a modification of a test system previously[3] described. The volume of enzyme solution added to the assay system was reduced by a factor of 10 (to 0.05 ml) and the time of reaction by a factor of 5 (to 1 min). These changes were made in order to avoid the otherwise necessary dilution steps and also in order to minimize any reactivation of the PCMB-inhibited enzyme in the assay system. Such a

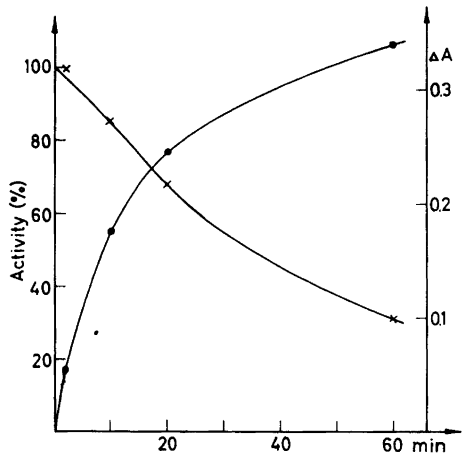

Fig. 1. Reaction between PCMB and rhodanese. Reaction conditions were PCMB 8.6×10^{-5} M, rhodanese 1.0×10^{-5} M, phosphate 0.05 M, pH 7.0 and temp. 23°C. • Increase in absorbancy at 250 mμ. X Rhodanese activity.

reactivation could be expected, as spectrophotometric measurements demonstrated that the rhodanese substrates, thiosulfate and cyanide, both reacted with an appreciable velocity with PCMB.

RESULTS

When native rhodanese was treated with PCMB at pH 7.0 a slow increase of absorbancy at 250 mμ was found (indicating a formation of PCMB-mercaptides) and at the same time the enzyme was inhibited (Fig. 1). The reaction was so slow, however, that a quantitative evaluation of the number of sulfhydryl groups present in the enzyme was impossible, as PCMB was not stable under the prolonged time periods necessary for complete reaction. At pH 4.6 an immediate turbidity developed which prevented any quantitative determinations at this pH. In the presence of 2 % dodecyl sulfate, however, a very rapid reaction (completed in less than 1 min) ensued at pH 7.0, which allowed the number of sulfhydryl groups in rhodanese to be determined according to the principles described by Boyer[4]. The unusual high molar absorbancy increment of 9.4×10^3 was obtained for rhodanese under these conditions. Determinations on two different samples of crystalline rhodanese gave values of 4.01 and 4.25 SH-groups per molecule of rhodanese.

The number of SH-groups in rhodanese was also determined by amperometric titration with silver ions. In the absence of any detergent only a change of slope of the titration curves without any detectable endpoint was observed. In the presence of 0.5 % dodecyl sulfate however, sharp and reproducible endpoints were obtained, corresponding to 4.35 and 4.57 SH-groups per molecule in satisfactory agreement with the values obtained in the experiments with PCMB. The fact that somewhat higher values are obtained by silver titration may be due to a binding of silver ions to other sites than sulfhydryl groups[6] in the protein.

DISCUSSION

Rhodanese shows in its reaction with PCMB a behaviour similar to that of other enzymes[7,8] which contain "masked" sulfhydryl groups. The fact that rhodanese activity decreases, when the sulfhydryl groups in the enzyme are blocked with PCMB, seems to support the interpretation that the sulfhydryl groups are "active sites" in the enzyme and participate in the enzymatic reaction. However, it has recently been shown[9–11] by measurements of optical rotatory dispersion that PCMB may induce changes in the tertiary structure of enzymes equivalent to denaturation, and such an effect could as well explain the results obtained with rhodanese. Of interest in this connection is the fact that crystalline rhodanese contains labile sulfur[12,13] and evidence has recently been obtained[13], which suggests that the labile sulfur is bound to a sulfhydryl group in the enzyme. This sulfhydryl group may be the only one, which participates in the enzymatic reaction, and may be protected from reaction with PCMB (and other sulfhydryl reagents) by the sulfur atom attached to it. PCMB could however react with the three other sulfhydryl groups present in the enzyme and the latter may then be inactivated by a secondary denaturation. It would be of interest to study the reaction between rhodanese and PCMB by optical dispersion measurements in order to further elucidate this point.

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