REACTION OF CACODYLIC ACID WITH ORGANIC THIOLS

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1. Introduction

Cacodylic acid is an arsenical with the structural formula $HOAsO(CH_3)_2$. Since its pK_a is 6.15, cacodylic acid is often used along with an appropriate cation to buffer enzyme-catalyzed reactions. We report here that cacodylic acid reacts with various SH-containing materials that are commonly used in enzyme-catalyzed reactions, such as 2-mercaptoethanol, cysteine, glutathione, and dithiothreitol. When cacodylate and these alkyl thiols are present together they may be expected to react spontaneously and thus interfere with the study of other reactions either by depleting the amount of these substances or by producing a product that is inhibitory.

2. Materials and methods

The chemicals used in this study were not purified beyond the quality obtained commercially. Free SH groups were measured quantitatively by the Ellman procedure [1] by adding 10–500 μl to 8 ml of a solution containing 0.02 M potassium phosphate pH 8.0 and 40 μg/ml of 5,5′-dithiobis(2-nitrobenzoic acid). The absorption at 412 nm was proportional to the SH concentration over the range 0–1 μmole; the absorption was about 20% higher in the presence of 1 mM cacodylate. Absorption measurements were made on a Gilford 2000 spectrophotometer, and spectra were obtained on a Cary 15 recording spectrophotometer. The temperature at which the reactions were studied was 24 ± 1°.

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3. Results

Fig. 1 demonstrates that 0.2 M 2-mercaptoethanol reacts with cacodylic acid at various concentrations so that the rate varies with concentration but the extent of loss of SH does not. When the molar concentration of cacodylate is equal to or greater than that of mercaptoethanol, the extent of the loss of SH groups reaches approx. 50% and does not proceed further, even when the cacodylate concentration is twice that of the 2-mercaptoethanol. Whether the reactive SH group remaining still represents 2-mercaptoethanol is not known.

The disappearance of the SH group is accompanied by a disappearance of the acid function of the cacodylic acid. In fig. 2 the amount of titratable acid is seen to decrease as the concentration of 2-mercaptoethanol is increased. Indeed, the titratable acid of the cacodylic may be virtually eliminated. Again, the maximum loss of SH is approx. 50%; when mercaptoethanol is in 10-fold excess, only 25% of the SH disappears. This may indicate that more than one SH reacts with one cacodylate. The mechanism of the reaction is apparently complex.

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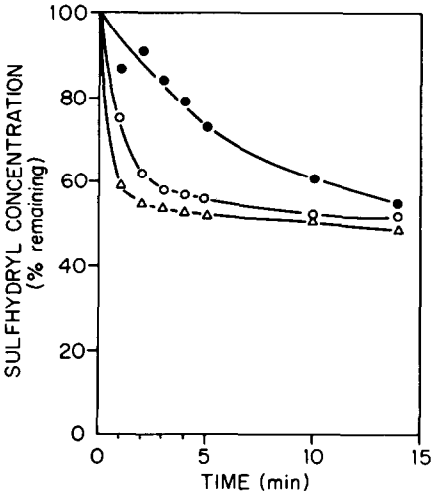

Fig. 1. Disappearance of SH groups in the presence of cacodylate. The amount of reactive SH remaining in a solution of 0.2 M 2-mercaptoethanol and 0.1 M (●—●—●), 0.2 M (○—○—○), and 0.4 M (△—△—△) potassium cacodylate pH 5.5 was determined by adding 10 μl to 8 ml of the Ellman assay mixture. The absorption at 412 nm at zero time was 1.652.

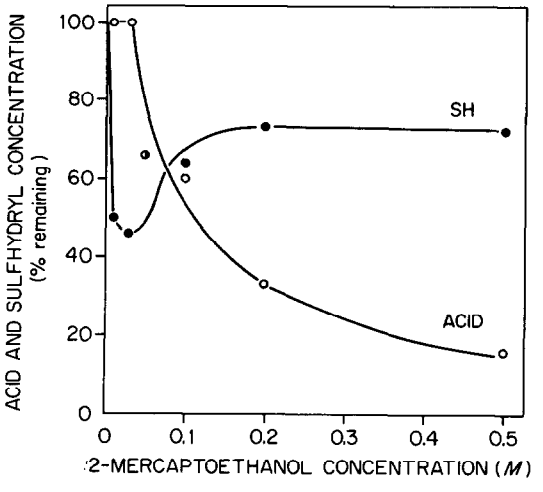

Fig. 2. Loss of titratable acid and of SH groups. The reaction mixture contained 50 mM potassium cacodylate pH 5.0, 10 mM potassium acetate pH 5.0, and 2-mercaptoethanol as shown. The amount of KOH required to adjust 10 ml of the reaction mixture to pH 7.0 was measured at 4, 12, and 30 min. SH concentration was determined as in fig. 1, using 10- to 500-μl samples, as appropriate. Values obtained at 30 min are presented since the reaction is complete at this time.

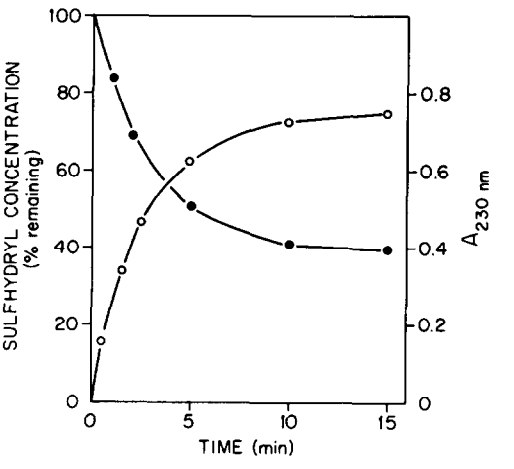

Fig. 3. Change in absorption at 230 nm correlated with disappearance of SH groups. The reaction mixture contained 50 mM 2-mercaptoethanol and 100 mM potassium cacodylate pH 5.5. Measurement of reactive SH was performed as described in fig. 1; measurement of A_{230} nm was made after diluting the sample 1/100 in water (○—○—○).

Some properties of the product of the reaction between 2-mercaptoethanol and cacodylate have been observed. A characteristic odor rather similar to the odor of HCN is produced when 50 mM cacodylate and 50 mM 2-mercaptoethanol react. As shown in fig. 3, an increase of the absorption at 230 nm parallels the disappearance of SH groups. If the reaction product is allowed to stand at pH 8.0 for 30 min, the absorption at 230 nm due to the product disappears. This disappearance is not the result of a simple reversal of the reaction, since no increase in measurable SH group concentration results from treatment at pH 8.0. In control experiments, 0.01 M 2-mercaptoethanol in 0.05 M acetate buffer pH 5.5 did not show a loss of SH group concentration, nor did there occur a time-dependent increase in absorption at 230 nm.

Other organic thiols also react with cacodylic acid. Solutions of L-cysteine, L--glutathione, and dithiothreitol, 50 mM each, were prepared in 50 and 500 mM potassium cacodylate pH 5.5. The disappearance of SH groups followed the same general time course as shown in fig. 1 for 2-mercaptoethanol, and a rise in the absorption at 230 nm also paralleled this decrease. In the case of dithiothreitol a white precipitate formed on mixing 0.05 M thiol with 0.5 M cacodylate pH 5.5.

Again, the controls showed that there was no loss of SH groups in the presence of acetate buffer pH 5.5. Since cacodylate reacts with cysteine and glutathione, the possibility arises that it may also react with the SH group of proteins. We do not as yet have evidence that supports this view, although is should be recognized as a possibility.

We have also considered the possibility that cacodylate reacts with sulfur-containing components of nucleic acids. For these studies the experimental approach was to see whether the spectrum of the nucleic acid components was perturbated by cacodylate, as compared to acetate at the same pH. There was no spectral alteration in the range 300–400 nm for *N*-methyl-4-thiouracil or for *E. coli* transfer RNA in cacodylate buffer. In the case of 4-thiouracil the sulfur exists almost exclusively as a thioketone, and thus it might not be expected to react.

From these observations we believe that the use of cacodylate buffer must be considered to interfere with the function of reducing agents such as 2-mercaptoethanol, glutathione, and dithiothreitol that are commonly used in enzyme-catalyzed reactions. In studies of the reaction of phenylalanyl-tRNA synthetase of *Neurospora crassa* with valine tRNA of *E. coli*, 2-mercaptoethanol was found to be an inhibitor of the reaction; the inhibition is much more severe in the presence of cacodylate than in the presence of Tris [2]. Presumably this is due to the presence of the reaction product of cacodylate and mercaptoethanol.

Cacodylate acid is in the arsenous class of compounds, with the arsenic in the 3^+ oxidation state. Another arsenous compound, sodium arsenite, has also been shown to react with dithiothreitol, as judged by the disappearance of the acid function and by changes in the infrared spectra; thus, cacodylic acid seems to be representative of the arsenous compounds, and the above observations may prove to be generally applicable. Further studies are being conducted to elucidate the identity of the product of the reaction of cacodylate with the organic thiols. Zahler and Cleland have reported that arsenite can be used as a reagent to determine the amount of dithiothreitol; they postulate that the two sulfurs are bound directly to arsenite [3] and find that the equilibrium constant is about 3. If the equilibrium of the reaction of 2-mercaptoethanol with cacodylate is also near unity, the limited disappearance of SH in fig. 1 may be understood.

References

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