## Dependence of the Function of Glucose Dehydrogenase of *Bacterium anitratum* on a Dissociable Cofactor

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It was previously reported that incubation of glucose dehydrogenase (GDH) of *B. anitratum* at room temperature for 1 h at pH 3.2 reduced the activity by 90 %, and that DPN or TPN failed to reactivate the enzyme [1]. We have now found that such a reactivation can be observed with boiled juice or with a neutralized perchloric acid extract of the enzyme. Under suitable conditions this reactivations is nearly complete.

Largely inactivated but reactivatable GDH preparations free from dissociated cofactor have been obtained in three ways: (*a*) by precipitation with acid ammonium sulfate, (*b*) by treatment with 0.003 N HCl followed by separation of the components by gel filtration, and (*c*) by freezing and thawing dilute neutral solutions in the presence of salts, again followed by gel filtration.

The reactivation requires a relatively concentrated mixture of apoenzyme and cofactor in order to proceed at a measurable rate. The dilution of the enzyme preparation as it is added to the assay mixture buffered at pH 6 may be some thousand fold, and addition of an acidified preparation directly gave less than 1 % of the activity observed when the acidified enzyme was first neutralized and then assayed. For routine studies of the reactivation a few microliters of apoenzyme was preincubated with a perchlorate extract in a small tube, and the mixture subsequently assayed for enzyme activity. Under these circumstances the reactivation was half maximal in 5 – 20 min at 20°C. The degree of reactivation at a given time increased toward a maximum with the amount of cofactor preparation added. In the presence of higher concentrations of salts the reconstitution was markedly inhibited.

Little is yet known about the chemical nature of the cofactor. From the behaviour in gel filtration it appears to have a molecular weight less than 1000. It exists as an anion at neutral pH, and its affinity for basic ion exchange resins should facilitate its further concentration and purification. The relationship of the cofactor to the group on the enzyme causing the absorption band at 337 mμ when the enzyme is reduced is not conclusively established. It is significant, however, that the dissociation of the factor from the enzyme is accompanied by a parallel disappearance of the 337 mμ band and of the enzyme activity.

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## Purification of an Inhibitor of Pancreatic Deoxyribonuclease

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Extracts from several different animal tissues contain a heat labile inhibitor of crystalline pancreatic DNase [1-2]. The inhibitor was obtained from calf spleen, other organs from the same animal having less activity per unit protein. It was purified 50-fold by ammonium sulfate fractionation, alumina hydroxide gel adsorption and chromatography on DEAE-Sephadex. At pH 7.5 the inhibitor was stabilized by mercaptoethanol and this substance was therefore added during the purification procedure.

DNase activity was assayed by a modification of a rapid spectrophotometric method [3]. The assay was made more sensitive by the addition of both $Mg^{2+}$ and $Ca^{2+}$ ions to the substrate [4]. Inhibitor activity was then measured as the difference between uninhibited and inhibited enzyme activity. The inhibitor showed the general properties of proteins with a stability optimum around pH 7.5 and a 260/280 ratio of 0.56 in the purest fractions. It was thermolabile and lost almost all activity on acid precipitation.

Preliminary studies of the mechanism of inhibition indicate that the inhibitor acts by being bound to DNase.

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