Solubilization and Properties of the Structurally Bound Glucose Dehydrogenase of *Bacterium anitratum*

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B. anitratum contains a soluble and a particulate glucose dehydrogenase, bound to the electron transport chain [1]. Studies of the structurally bound dehydrogenase while still attached to the particles revealed a close resemblance to the soluble enzyme, and led to the postulate that the latter either is a precursor or a degradation product of the particle-bound enzyme. In order to establish more firmly this apparent identity, it was necessary to obtain the bound enzyme in a solubilized state.

Several means of solubilization of the enzyme from the large particles of the cell homogenate were tried, including sonic disruption, treatment with organic solvents, various snake venoms, and trypsin. These were all without appreciable effect. However, by "dissolving" the thoroughly washed particles at pH 7.5 in the presence of 2.2 mg desoxycholate per mg protein, and fractionating this "solution" with protamine sulfate and ammonium sulfate, 68 % of the activity precipitated between 61 and 76 % saturation, the same concentration of salt that also precipitates the soluble enzyme. The majority of the enzyme activity of this fraction failed to become bound to DEAE-cellulose at pH 7, and was finally concentrated by absorption on and elution from CM-cellulose, again behaving in conformity with the soluble enzyme of the cell. The overall apparent purification was 270-fold, with an apparent yield of 50 %.

Upon addition of glucose to the preparation, an absorption band with a maximum at 337 mμ appeared. This is the most characteristic feature of the soluble enzyme of the cell [2]. The originally soluble enzyme also has a characteristic specificity pattern observable with 0.02 M solutions of different aldoses [3]. The very same pattern was observed with the solubilized dehydrogenase, while it, because of lower apparent Michaelis constants, was partially masked with 0.02 M solutions for the enzyme still in the particle.

The final preparation appears to represent the enzyme in true solution, and not as a subunit of the original particle kept suspended by desoxycholate. The behaviour in the purification process suggests this to be the case, and assay for desoxycholate revealed the presence of not more than 0.001 mg per mg protein. The absorption spectrum revealed only traces of the hemoproteins known to be associated with the enzyme in the particle.

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Co-factor Requirements for Phosphodeoxyribomutase from Calf Thymus

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Phosphodeoxyribomutase converts deoxyribose 1-phosphate to deoxyribose 5-phosphate [1]. Studies on this reaction indicated the requirement of an activator for maximal reaction rate [2]. The activator was present as an impurity in the substrate deoxyribose 1-phosphate which had been prepared from thymidine and phosphate using horse liver phosphorylase. The activator could be separated from the substrate by electrophoresis. It had the same mobility as ribose 1,5-diphosphate but was not identified. The suggestion was made that the activation was due to small amounts of deoxyribose 1,5-diphosphate being present in the substrate preparation.

The experiments described in the present communication were made to elucidate further the requirements for activator in the phosphodeoxyribomutase reaction and to provide further support for the presence of deoxyribose 1,5-diphosphate in the substrate. Calf thymus