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

Per A. Hallberg and Jens G. Hauge

National Institute of Public Health, Oslo, Norway

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

J. Jonsen, S. Laland and N. O. Solum

Department of Biochemistry, University of Oslo, Blindern, Norway

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

was used as source of the enzyme. The conversion of deoxyribose 1-phosphate to deoxyribose 5-phosphate was followed using a method described previously [3]. It was found that the rate of conversion of synthetic deoxyribose 1-phosphate was nearly identical to that obtained with the enzymatically prepared substrate. The necessity for an activator for optimal reaction rate was thus not confirmed. In order to account for the apparent discrepancies in the two sets of experiments it was assumed that variations in the procedures involved in the preparation of phosphodeoxyribomutase might result in two different forms of the enzyme: one phospho-enzyme acting without activator or another dephospho-enzyme which required a sugar 1,5-diphosphate as cofactor. The above assumption was tested by studying the phosphodeoxyribomutase reaction in the presence of ^{32}P added as orthophosphate and using the non-activator requiring enzyme. The deoxyribose 5-phosphate formed was isolated by paper chromatography and it was shown to contain significant amounts of radioactive phosphate. This indicated that ^{32}P had been incorporated into the enzyme followed by transfer of the radioactive phosphorus from the phospho-enzyme with the formation of deoxyribose 5-phosphate. The phosphodeoxyribomutase reaction would then be analogous to the phosphoribomutase reaction as proposed by Klenow [4].

When deoxyribose 1-phosphate was enzymatically prepared in the presence of ^{32}P, radioactivity was also detected in a substance which had the same mobility as ribose 1,5-diphosphate by electrophoresis. Furthermore treatment at room temperature with an acetate buffer pH 4.0 converted as expected the substance to a radioactive substance which had the same chromatographic mobility as deoxyribose 5-phosphate. It is therefore assumed that deoxyribose 1,5-diphosphate is formed from deoxyribose 1-phosphate by a deoxyribose 1-phosphate transphosphorylase present in the liver phosphorylase which was used for the preparation of deoxyribose 1-phosphate.

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Assay of Aldolase Activity Utilizing the 3-Methyl-2-benzothiazolone Hydrazone Test

Egil Jellum

The Research Laboratory, Lier Hospital, Lier, Norway

A new reagent, 3-methyl-2-benzothiazolone hydrazone (MBTH) has recently been introduced in quantitative photometry for the determination of aliphatic aldehydes [1], carbazoles, azodyes, stilbenes, aromatic amines [2] and tetrapyrrols like bilirubin and biliverdin [3].

The reaction with aliphatic aldehydes includes the following three steps: (1) Condensation between MBTH and the aldehyde to form a colorless product (an azine). (2) Oxidation of excess MBTH by ferric chloride in acid media to form a reactive cation. (3) Condensation between the two products to form a highly colored cationic pigment.

The MBTH test, probably the most sensitive method yet known for detection of aliphatic aldehydes [1], gives, when applied to glyceraldehyde phosphate, a highly colored blue pigment with a broad absorption maximum at 610 mμ and a molar extinction coefficient exceeding 50 000. Based on this finding, a new method has been developed for the assay of aldolase activity.

A mixture of fructose 1,6-diphosphate, veronal buffer, MBTH and the enzyme, *e.g.* hemolysed red cells, is incubated at 37°, pH 8.6, for 15 min, and the liberated triosephosphates are trapped by the MBTH as they are being formed (step 1). The reaction is terminated by the addition of TCA, and after removal of the proteins, aliquots of the TCA extract are diluted and ferric chloride is added. A blue color is slowly produced (steps 2 and 3) and can be read in a spectrophotometer at 610 mμ after 30 min at room temperature. The color, which is stable for at least 2 h, is proportional to the amount of glyceraldehyde phosphate liberated in the reaction.

The advantages of the new MBTH method are simplicity and sensitivity. The simplicity makes the procedure particularly suited for routine analyses. The sensitivity, if fully exploited, may be used to determine minute amounts of aldolase or to reduce the incubation time considerably. Thus, if required, the incubation time, using hemolysed red cells as enzyme, may be reduced to 3 min. The dis-