THE EFFECT OF p-HYDROXYMERCURIBENZOATE AND CONGENERS ON MICROSMAL GLUCOSE-6-PHOSPHATASE*

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(Received October 30, 1975)

Summary

Iodoacetamide, N-ethylmaleimide, p-hydroxymercuribenzoate (p-MB) and HgCl₂ were tested as inhibitors of microsomal glucose-6-phosphatase. Iodoacetamide had no effect at 2 mM. N-ethylmaleimide inhibited only crude, but not purified microsomal preparations (M₂) or crude microsomes exposed to deoxycholate. ¹⁴C-labelled N-ethylmaleimide was not bound by the M₂ protein fraction. p-MB inhibited all types of preparations and the inhibition was not counteracted by detergent. A more detailed study was carried out with the purified M₂ fraction (specific activity: 2-4 μmoles Pᵢ/min/mg protein). Glucose-6-phosphate hydrolysis was inhibited 50% by 5 × 10⁻⁵ M p-MB. The inhibition was completely reversible by dithiothreitol except when the enzyme was pre-incubated with p-MB in the absence of substrate. Then p-MB accelerated the temperature-dependent inactivation of glucose-6-phosphatase. Binding studies showed that around 3 μmoles ¹⁴C-p-MB were incorporated into 100 mg M₂ protein regardless of the concentration of mercurial in the incubation mixture. That is, over a 25 fold range of p-MB concentration, causing up to 80% inhibition of enzyme activity, no difference was seen in the amount of labelled p-MB which was irreversibly bound to M₂ protein. Kinetically p-MB behaved like a reversible inhibitor and this was confirmed by dilution experiments. Several compounds, including some amino acids, antagonized the inhibition by p-MB. The order of effectiveness was EDTA > barbital > tryptophan > histidine > lysine > other amino acids. Glycine, Tris and urea were ineffective competitors of p-MB inhibition. Double reciprocal plots showed that the Kᵅ for glucose-6-phosphate was increased and the Vₘₐₓ reduced in the presence of p-MB. HgCl₂ was a more effective inhibitor than p-MB with a Kᵅ of 6 × 10⁻⁶ M. We conclude that a reaction of p-MB with M₂ sulfhydryls does not play a part in the inhibition of enzyme activity. It is suggested that p-MB may interact with one or more amino acid side chains in such a way that enzyme conformation is altered.

Introduction

This study is part of a larger program designed to explore the functional role of glucose-6-phosphatase as a membrane-bound enzyme of the endoplasmic reticulum of liver and kidney. The main function of this enzyme appears to be blood sugar formation from glucose-6-phosphate, but it is not clear how glucose-6-phosphate formed by hexokinase can bypass this enzyme on its way to glycogen. No regulatory control has been reported so far. One is therefore left with the notion that the special location of the enzyme has something to do
with the separation of the same intermediate as part of an anabolic or catabolic pathway.

The genetically determined loss of glucose-6-phosphatase in type I glycogen storage disease in humans is well known. Recently, in mice, four X-ray induced albino mutants have been shown to develop hypoglycemia immediately after birth and to lack glucose-6-phosphatase in liver and kidney. There were ultrastructural abnormalities of the endoplasmic reticulum in these two tissues that normally contain glucose-6-phosphatase, but not in other tissues of the albino mutants. This suggests that glucose-6-phosphatase where it occurs is an integral part of the membrane structure.

Attempts to solubilize the enzyme in an active form have not so far been successful. In fact, considerable purification of the enzyme could be achieved by differential extraction of other proteins from the microsomal particles. The present study was undertaken with such purified particles that show the following characteristics. The specific activity is about 20 times higher than in crude microsomal particles. The phospholipid content of the particles is reduced but not to the point where addition of phospholipid is required to restore full activity. Latent enzyme activity, detectable by addition of deoxycholate or other detergents, is absent. These purified particles, termed M2, still contain several proteins when solubilized in 1% sodium dodecyl sulfate and examined by acrylamide gel electrophoresis. It was hoped to identify the enzyme on such gels by labelling it with a radioactive sulfhydryl reagent during different stages of purification and to make use of the mutant mouse livers in which enzyme activity could not be detected. It was also known from previous work that the enzyme is inhibited by p-hydroxymercuribenzoate (p-MB)*. Although this project has not been successful, a number of other observations have been made on the activity of this membrane-bound enzyme as influenced by sulfhydryl reagents.

Materials and Methods

Preparations of particulate M and M2 fractions, the former a lyophylized microsomal fraction and the latter a more purified and partly delipidated fraction, were made as previously described. Enzyme activity tests were for 20 minutes at 30° with 0.04 M glucose-6-phosphate, pH 6.8, in a volume of 0.5 ml unless otherwise stated. 0.5 ml of a 4% perchloric acid solution 10% in trichloroacetic acid was added to terminate the reaction. Specific activity is given in μmoles inorganic P formed per mg of protein per minute at 30°. Inorganic P was determined by the Fiske-Subbarow method and protein by the Lowry et al., method with bovine serum albumin as standard. For the determination of phospholipid P the samples were ashed and analyzed by the Ames method. The latter method, being about 100 times more sensitive than the Fiske-Subbarow method, was also used for the determination of the Michaelis constant (Km). Depletion of substrate at low concentration was thereby avoided.

The source of bile salts, phospholipids and glucose-6-phosphate has been given previously. p-MB as the sodium salt was obtained from the Aldrich Chemical Co. Carboxyl-labelled 14C-p-MB as the chloride, 10.1 μCi per μmole, was obtained from ICN (International Chemical and Nuclear Corporation). A slight excess of NaOH was used to dissolve the compound and aliquots were dried and kept in vacuo in the dark until used. The concentration of each p-MB solution was determined at 233 mμ, using 1.694 x 10^4 as the molar extinction coefficient. Iodoacetamide was from Aldrich. Unlabelled N-ethylmaleimide was obtained from Sigma and 14C-labelled NEM from New England Nuclear.

In preliminary experiments sulfhydryl groups of M preparations were titrated with p-MB according to the method of Boyer. 0.5 mg of protein was sonicated in 1 ml of 0.01 M phosphate buffer, pH 7 containing 0.4% deoxycholate or 1.0% sodium dodecyl sulfate and titrated with 0.01 ml increments of 8 x 10^-4 M p-MB and read against the same protein solutions as blank. M2 preparations could not be titrated successfully because of development of turbidity.

In experiments with 14C-p-MB 0.1 to 0.3 mg of M2 protein in 1 ml of 0.02 M citrate pH 6.8 was exposed to various concentrations of p-MB containing about 0.5 μCi of labelled mercurial. At the end of incubation proteins were precipitated with 9 volumes of acetone. Non-covalently