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DETERMINATION OF GLUCOKINASE AND HEXOKINASE ACTIVITY IN LIVER EXTRACTS PREVIOUSLY PURIFIED ON MOLSELECT G-50 DEXTRAN GEL

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Hexokinase and glucokinase activity in the supernatant of a rabbit liver homogenate obtained at 18,000g was determined by a spectrophotometric method. Preliminary purification to remove low-molecular-weight components by gel filtration on Molselect G-50 dextran was shown to prevent reduction of NADP unconnected with the hexokinase reaction.

KEY WORDS: hexokinase; glucokinase; rabbit liver; gel filtration; method of determination of enzyme activity.

The spectrophotometric method of determination of glucokinase (GK) and hexokinase (HK) activity of the liver, based on recording the increase in optical density at 340 nm due to reduction of NADP in the presence of an excess of glucose-6-phosphate dehydrogenase, is widely used at the present time [4, 5, 8-13]. The method requires preliminary purification of the liver extract by dialysis from low-molecular-weight components [4]. However, prolonged analysis can lead to considerable inactivation of the enzymes [12]. The gel-filtration method has considerable advantages over dialysis.

This paper describes an investigation of the effectiveness of preliminary purification of liver extract by gel filtration on Molselect G-50 dextran during determination of liver hexokinase activity.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 2.5-3 kg. To determine GK and HK activity the supernatant obtained by centrifugation of the liver homogenate at 18,000g and at 0-2° C for 1 h was used. The supernatant was purified on Molselect G-50 dextran gel (Reanal, Hungary) [13]. Fractions containing protein and free from low-molecular-weight compounds were used to determine the liver hexokinase activity. Activity of GK and HK was determined by a spectrophotometric method [10]. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

Typical curves characterizing the rate of reduction of NADP are given in Figs. 1-3. On addition of different amounts of protein of the unpurified liver supernatant to the incubation medium, the rate of reduction of NADP was not directly proportional to the quantity of protein added (Fig. 1). The reaction of NADP reduction on the addition of unpurified supernatant took place fairly intensively if the incubation medium did not contain ATP and glucose, which are necessary for the hexokinase reaction (Fig. 2). Under those conditions, when the spectrophotometric method was used to determine GK and HK activity, considerable deviations from the true value took place. It was shown that during determination of the GK and HK activity of the purified liver supernatant the rate of NADP reduction was directly proportional to the quantity of added protein of the eluate (Fig. 1). No reduction of NADP took place on the addition of purified liver supernatant to incubation medium without ATP or glucose (Fig. 3).

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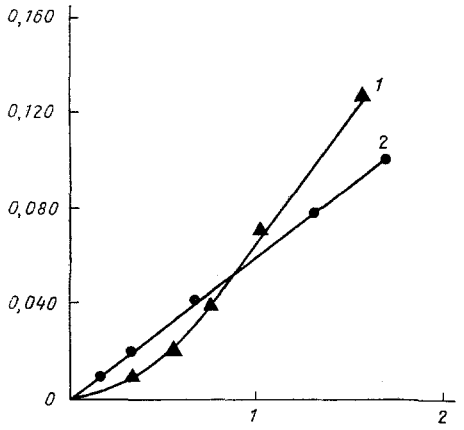

Fig. 1

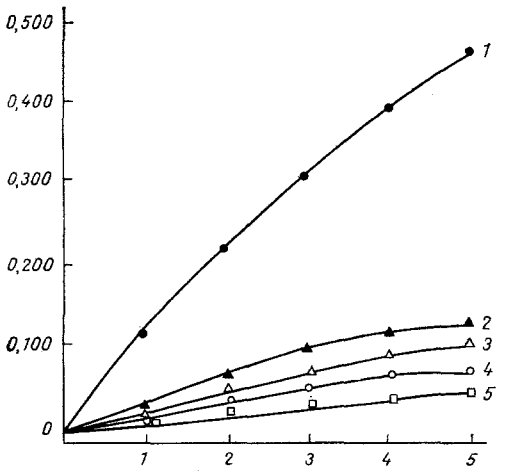

Fig. 2

Fig. 1. Rate of reduction of NADP as a function of quantity of added protein of soluble fraction of liver cytoplasm. Abscissa, protein content (in mg); ordinate, change in optical density at 340 nm in 1 min. 1) Addition of unpurified liver supernatant; 2) addition of purified liver supernatant.

Fig. 2. Rate of reduction of NADP as a function of composition of incubation medium during investigation of unpurified soluble fractions of liver cytoplasm. Abscissa, time (in min); ordinate, optical density at 340 nm. 1) incubation medium contained 100 mM glucose; 2) 0.5 mM glucose; 3) 100 mM glucose without ATP; 4) incubation medium without glucose; 5) 0.5 mM glucose without ATP.

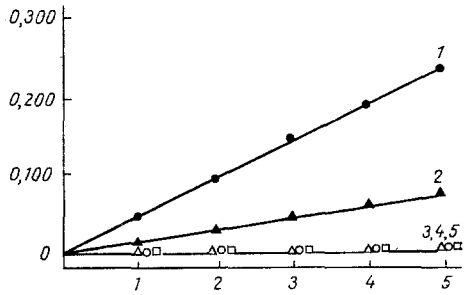

Fig. 3. Rate of reduction of NADP as a function of composition of incubation medium during investigation of purified soluble fraction of liver cytoplasm. Legend as in Fig. 2.

The experiments first showed that the use of preliminary purification of the liver extracts by gel filtration on Molselect G-50 dextran enables the activity of the enzymes to be determined much more accurately and quickly. The simplicity of the procedure for purification of the liver extracts by gel filtration on Molselect G-50 dextran is such that this method can be recommended for wide use in laboratory practice.

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