THE INHIBITION OF GLUCOSE-6-PHOSPHATASE BY PHLORIZIN AND STRUCTURALLY RELATED COMPOUNDS

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The glucoside phlorizin, best known as an inhibitor of glucose transport, is also an inhibitor of the enzyme glucose-6-phosphatase (Broh-Khan and Mirsky, 1948). The inhibition has been described as competitive by Ashmore (Ashmore and Weber, 1959), and as non competitive by Soodsma et al. (1967).

This report describes the results obtained when a series of compounds related to phlorizin were tested for inhibition. The most effective inhibitor was phloretin, the aglucone of phlorizin, while phlorizin and phloroacetophenone were weaker inhibitors. The inhibition was competitive for the enzyme from liver, but not for the enzyme from kidney.

Materials and Methods

The microsomal fraction from rat liver was used as a source of enzyme. Tissues were homogenized in eight volumes of 0.25 M sucrose containing 0.001 M EDTA, pH 7.4. The homogenate was centrifuged for ten minutes at 13,000 x g, and the supernatant fluid carefully removed with a pipette and centrifuged at 100,000 x g for 45 minutes. The pellet was centrifuged down once more and suspended in sucrose-EDTA before being frozen for storage.

Enzyme activity was measured by determining release of phosphate from glucose-6-phosphate. The assay medium was 0.05 M histidine, pH 6.2, containing 0.005 M EDTA. Incubations were for 20 minutes at 37 degrees.

Phosphate was determined by the method of Gomori (1942), and protein by the method of Lowry et al. (1951).

All compounds possessing inhibitory activity were recrystallized twice from ethanol before use. Since phloretin is only slightly soluble in water, it was ground with a mortar and pestle before being taken up in water to form an opalescent suspension. It was pipetted in this form.

Results

The inhibition of glucose-6-phosphatase by phloretin, shown in Fig. 1, is clearly competitive. The K_i value was 4.4×10^{-5} M. Plots of 1/v versus I (Dixon, 1953) yielded a curved line which approached a plateau. This probably reflects the low solubility of this compound.

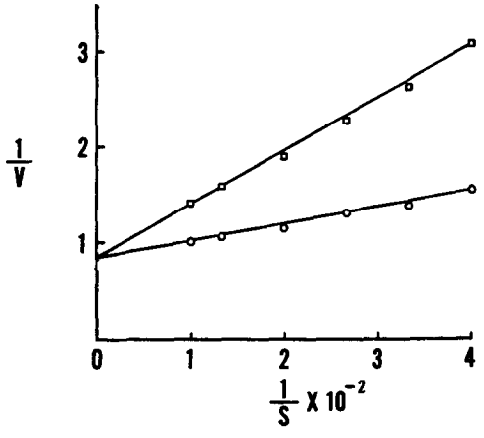

Fig. 1 - Inhibition of glucose-6-phosphatase by phloretin. Substrate is given as glucose-6-phosphate molarity, and velocity is expressed as the μmoles of phosphate released. Conditions were as described in the text. Each tube contained 0.42 mg of protein and: 0, no inhibitor; □, 1.0×10^{-4} M phloretin.

As shown in Figs. 2 and 3, the enzyme was also inhibited competitively by phlorizin and phloroacetophenone. The K_i values for these inhibitors were 3.8×10^{-4} M and 6.5×10^{-4} M respectively. The K_m values for glucose-6-phosphate in the experiments of Figs. 1 to 3 varied between 1.5 to 2.0×10^{-3} M.

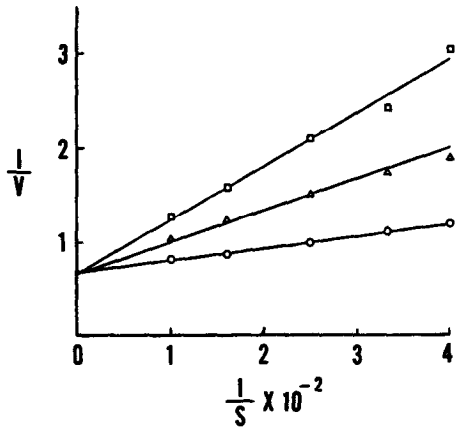

Fig. 2 - Inhibition of glucose-6-phosphatase by phlorizin. Substrate is given as glucose-6-phosphate molarity, and the velocity is expressed as the μmoles of phosphate released. Conditions were as described in the text. Each tube contained 0.26 mg of protein and: 0, no inhibitor; □ 1.25×10^{-3} M phlorizin; Δ, 6.25×10^{-4} M phlorizin.

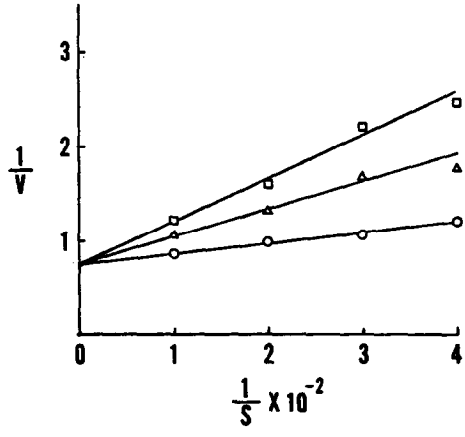

Fig. 3 - Inhibition of glucose-6-phosphatase by phloroacetophenone. Substrate is given as glucose-6-phosphate molarity, and velocity is expressed as the μmoles of phosphate released. Conditions were as described in the text. Each tube contained 0.26 mg of protein and: 0, no inhibitor; , □ 2.0×10^{-3} M acetophenone; Δ, 1.0×10^{-3} M phloroacetophenone.

While this work was in progress, Soodsma et al. (1967) showed that phlorizin inhibition was non competitive in kidney preparations. This behavior seems to depend on the source of the enzyme, since similar re-

sults were obtained in this laboratory if kidney enzyme was used in place of liver enzyme. This was true both at pH 6.2 and at the pH of 5.5 used by Soodsma and his coworkers. However, certain conditions which could not be consistently reproduced did introduce a non competitive component to the inhibition of the liver enzyme. The absence of EDTA, or use of older enzyme preparations, especially at pH 5.5, are examples of this. It appears that changes in the enzyme may sometimes alter the nature of the inhibition.

A variety of compounds with a structural relationship to phlorizin failed to inhibit. This included 0.01 M levels of phloroglucinol, arbutin (the glucoside of hydroquinone), and salicin (the glucoside of salicyl alcohol). Saturated solutions of the flavones quercitin and hesperidin also failed to inhibit, although quercitrin (the glycoside of quercitin) did inhibit slightly.

Discussion

Competitive inhibitors are assumed to bind at the active sites of enzymes because they are attracted by the same forces which attract the substrate. The inhibition reported above must be highly specific for phloretin since there is a ten fold increase in K_i when phloretin is altered by removal of the second phenolic ring (phloroacetophenone), or by formation of the glucoside (phlorizin). Therefore, it is puzzling to find that the molecular model of phloretin does not possess a particularly strong resemblance to that of glucose-6-phosphate.

There is no established connection between the inhibition of glucose-6-phosphatase and that of sugar transport across membranes. However, it is interesting to note that Soodsma et al. (1967) found that phlorizin would not inhibit glucose-6-phosphatase after the microsomal structure had been destroyed by deoxycholate. The existence of a dissociable phlorizin receptor was considered by these investigators. At present, it can only be concluded that phloretin and its congeners are

a very unusual group of inhibitors.

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