## The lack of insulin-binding to simple glycosidic receptors

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*Summary.* The results of affinity electrophoresis at pH 8.9 show that insulin does not interact with any of a number of glycosidic ligands which bind various lectins. The results are discussed with respect to some similarities between insulin and concanavalin A.

Concanavalin A competes with insulin for receptors on the surface of the brown fat cells[1] and exerts insulin-like effects. The insulin receptors are probably of carbohydrate nature[2], similar to the lectin receptors. Moreover, insulin was reported to bind free D-glucose with an association constant similar to the lectin-sugar complexes[3]. Therefore, it was tempting to study the possible interaction of insulin with simple immobilized glycosidic receptors which bind effectively concanavalin A and similar lectins. We have tested this presumed interaction by our modification of affinity electrophoresis[4] which makes it possible to detect even very weak interactions of a protein with the immobilized ligand and to test a wide range of potential receptors. The method is based on addition of water-soluble synthetic O-glycosyl polyacrylamide copolymers (or other suitable macromolecular substance containing covalently bound the ligand under study) into the polymerization mixture used for preparation of ordinary gel rods for disc gel electrophoresis. Mobility of a protein on a gel with the immobilized ligand decreases with increasing concentration of the immobilized ligand. Introduction of a free ligand into this affinity gel leads to a reversion of the effect of the immobilized ligand due to the competition of free and immobilized ligand for the ligand-binding site in the protein. At sufficiently high concentration of the free ligand, the protein has a mobility almost equal to that on control gel (i.e. the gel without any immobilized ligand). In this way we have tested a variety of different O-glycosyl polyacrylamide copolymers for their interaction with insulin.

*Materials and methods.* Insulin (Serva) contained 0.6% of Zn. O-glycosyl polyacrylamide copolymers were prepared by copolymerization of water solutions of acrylamide and allyl glycosides of the sugars studied, as described in detail previously[5].

The following copolymers were used (in parantheses is their sugar content): D-ribosyl- (16.4%), D-arabinosyl- (14.7%), L-arabinosyl- (11.7%), D-xylosyl- (15.6%), D-lyxosyl- (18.0%), α-D-glucosyl- (20.8%), β-D-glucosyl- (11.5%), α-D-mannosyl- (15.0%), α-D-galactosyl- (8.6%), α-L-fucosyl- (16.3%), L-rhamnosyl- (11.3%), N-acetyl-α-D-galactosaminyl- (3.3%), N-acetyl-α-D-glucosaminyl- (4.8%), N-acetyl-β-D-glucosaminyl- (6.6%), β-lactosyl- (15.6%), β-cellobiosyl- (15.0%), β-maltosyl- (14.0%). The general procedures used for preparation of α- and β-glycosides have been described elsewhere[6]. In the cases where the anomeric configuration (α or β) is not indicated, the sirupy mixture obtained as a result of Fischer's reaction[7], after evaporation of allyl alcohol and containing admixtures of β-glycosides and remaining unreacted free sugar (about 20–50%), was used directly for copolymerization without prior isolation of the crystalline glycoside.

Disc polyacrylamide gel electrophoresis in an alkaline buffer system[8] was performed in an apparatus designed by Davis[9] using gel rod dimensions 70 × 5 mm (omitting the large pore gel layer), sample size 50 μg in 20 μl of 20% glycerol solution and current density 5 mA per tube. The electrophoresis was run until bromphenol blue (a tracing dye) reached the bottom of the gel. Affinity electrophoresis was carried out under the same conditions, using 2 concentrations of the copolymer (0.5% and 1%, respectively) without or with 0.1 M free sugar. Insulin bands were visualized by immersion of the gels into 10% trichloroacetic acid after the electrophoresis.

*Results and discussion.* Mobility of the main insulin zone was nearly the same as the mobility of bromphenol blue. Moreover, at least 3 slower minor bands were observed, corresponding probably to associated species. Thus, insulin possessed ideal electrophoretic properties for the study by affinity electrophoresis. Despite this fact, no retardation was observed on affinity gels, either at 0.5% or 1% concentrations of the copolymers given in the Materials and methods section, as compared with the control gels (containing no copolymer). Only in the cases of cellobiose, D-arabinose and N-acetyl-D-glucosamine containing copolymers a slight retardation (by about 5% of the total mobility) was observed at 1% copolymer concentration, but this retardation could not be abolished by addition of the corresponding free sugar in final concentration 0.1 M. This slight retardation was probably due to a nonspecific effect of a high concentration of the copolymer. Thus, we were not able to detect any interaction of the glycosidically bound sugars with insulin; under the conditions of our study, an interaction characterized by $K_i$ (dissociation constant of the complex insulin-immobilized sugar) as high as about $5 \cdot 10^{-1}$ M would clearly be detected in most cases. It should be noted that interaction of many lectins with O-glycosyl polyacrylamide copolymers is very strong and easily detectable under similar conditions[4].

Our results are rather surprising when we consider the observations of Anzenbacher and Kalous[3], who found on the basis of equilibrium dialysis binding of free D-glucose to insulin. The difference in results may be explained in 2 ways: 1. In the experiments of these authors, insulin may have interacted with the free anomeric hydroxyl of D-glucose not available in our copolymers. The authors did not study binding of low molecular D-glucosides. 2. Insulin may require a longer spacer than the methylene group present in the O-glycosyl polyacrylamide copolymers used by us. To test this possibility, it would be necessary to synthesize soluble macromolecular derivatives with the sugar bound over a longer hydrophilic chain.

Our experiments show that insulin, in contrast to lectins, is probably not able to bind to simple immobilized sugar receptors. It might be interesting to immobilize some insulin-receptor active material and to study by affinity electrophoresis its interaction with insulin and the possible inhibition of the interaction with different soluble carbohydrates.

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