Kinetic, chromatographic and electrophoretic studies on glucose-phosphorylating enzymes of rat intestinal mucosa

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The number and nature of glucose-phosphorylating enzymes of rat intestinal mucosa were investigated by chromatographic, electrophoretic, and kinetic methods. Three fractions with glucose-phosphorylating activity were obtained from the supernatant fluid of mucosa homogenate by means of DEAE-cellulose chromatography, corresponding to hexokinases A and B (EC 2.7.1.1.), and N-acetyl-d-glucosamine kinase (EC 2.7.1.59). Although the latter uses N-acetylglucosamine as the main substrate, it is also able to phosphorylate glucose. Electrophoresis in polyacrylamide and in cellulose acetate gels showed the same three enzyme activities. None of these procedures revealed the presence of either hexokinase D ("glucokinase") or hexokinase C in the intestinal mucosa. In the sediment fractions hexokinase A and B, but not N-acetylglucosamine kinase, were found. The Km values for glucose of partially purified hexokinases A and B were 0.025 and 0.174 mm, respectively, and their substrate specificity was th