EVIDENCE FOR AN INTERACTION BETWEEN KIDNEY CELL MEMBRANE AND CERTAIN PHOSPHATE-TRANSFERRING ENZYMES

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As a facet of our investigation of the role of adenosine-3',5'-monophosphate in the mechanism of action of antidiuretic hormone we have explored the possibility of an interaction between kidney cell membrane and certain phosphate-transferring enzymes. This communication is concerned with evidence for a membrane-enzyme interaction as derived from optical density studies, enzyme activation and inhibition effects, and optical rotatory dispersion data. We have found that phosphoglucomutase, phosphorylase, and pyruvic kinase appear to interact with kidney cell membrane. These enzymes are unique with respect to those studied in requiring a substrate which bears a phosphorylated carbonyl moiety.

Materials and Methods

Optical density measurements were performed using a Beckman DB Spectrophotometer with linear-logarithmic potentiometric recorder.

Phosphorylase was determined according to the method of Helmreich and Cori (1964). In order to determine if membrane could serve as a substrate for phosphorylase with the concomitant production of glucose-1-phosphate, endoplasmic reticulum was substituted for omitted glycogen. In order to obtain evidence for membrane phosphorolysis without glucose-1-phosphate production, phosphorylase was added to a suspension of membrane, aliquots

were removed at 30 seconds, 10 minutes, and 20 minutes and each was rapidly introduced into the complete assay medium. Phosphoglucomutase was determined by modifying the phosphorylase assay medium through omission of glycogen and adenosine-5'-phosphate and addition of 4×10^{-3} M glucose-1-phosphate. Pyruvic kinase was determined according to the procedure of Bucher and Pfleiderer (1955). Each of these enzymic activity determinations depends upon the production of or the destruction of a reduced pyridine nucleotide and, thus, the slope of the recorded optical density at 340 mμ depicted the enzyme activity.

Optical rotatory dispersion studies were performed using a Cary Model 60 Recording Spectropolarimeter equipped with a jacketed cell of 1 cm path length. Data were obtained using a period of 3, full range of 0.2°, and with programmed slits. Solutions were adjusted to pH 7.47 - 7.48. Enzyme concentrations were 40 μg/ml. Cell membrane concentrations were those which provided an optical density of approximately 0.8 at 210 mμ.

Phosphorylase, apyrase, and neuraminidase were obtained from Sigma Chemical Company of St. Louis, Missouri. Hexokinase, phosphoglucomutase, creatine phosphokinase, alkaline phosphatase, and pyruvic kinase were obtained from C. F. Boehringer and Soehn of Mannheim, Germany, distributed by California Corporation for Biochemical Research, Los Angeles, California. Hyaluronidase and the adenosine phosphates were also obtained from this vendor. Arginine vasopressin was synthesized by Sandoz Incorporated of Switzerland and distributed by the Pharmaceutical Division of Sandoz, Incorporated, Hanover, New Jersey.

Canine kidney endoplasmic reticulum was isolated from the cortical portion according to the procedure of Landon and Norris (1963). Cell membrane was isolated from canine cortical tissue

according to the procedure of Neville (1960). Each of the membraneous preparations was observed under a Leitz microscope equipped for phase contrast.

Results and Discussion

The addition of 250 µg of phosphoglucomutase, of phosphorylase, or of pyruvic kinase to 2 ml of a cell membrane suspension resulted in an increase in optical density over and above that of the sum of the components. Similar addition of creatine phosphokinase, apyrase, alkaline phosphatase, hexokinase, neuraminidase, or hyaluronidase did not produce an anomalous optical density. The addition of 10^{-5} M adenosine triphosphate or adenosine diphosphate, or of 10^{-6} M adenosine-3',5'-monophosphate, or of 10^{-7} M arginine vasopressin failed to alter the optical density of kidney cell membrane suspensions. Data for mixtures which exhibited an anomalous optical density are presented in Table I.

TABLE I

ANOMALOUS INCREASE IN OPTICAL DENSITY RESULTING FROM MEMBRANE-ENZYME INTERACTION

A 250 µg aliquot of enzyme was added to 2.0 ml of membrane suspension subsequent to obtaining the optical density of each separately.

MEMBRANE PREPARATION	ENZYME	SUM OF COMPONENT OPTICAL DENSITIES	OBSERVED OPTICAL DENSITY	PERCENTAGE INCREASE
isolated cell membrane	phosphoglucomutase	0.120	0.160	33
isolated cell membrane	phosphorylase	0.125	0.150	20
endoplasmic reticulum	phosphorylase	0.209	0.309	48
isolated cell membrane	pyruvic kinase	0.120	0.140	17

The interaction between kidney cell membrane and these enzymes would appear to involve binding or aggregating since an increase in optical density generally reflects an increase in particle size or density. Of the nine enzymes which were employed in these studies the three which induce anomalous optical density effects, phosphoglucomutase, phosphorylase, and pyruvic kinase, are unique in requiring a substrate which bears a phosphorylated carbonyl moiety, i. e., glucose-1-phosphate or phosphoenolpyruvate. It is suggested that these enzymes each possess a specific type of binding site which finds a suitable membrane substituent with which to interact.

We then undertook to learn if interaction between kidney cell membrane and enzyme involved the membrane functioning as a substrate. Since cell membrane has been reported to possess a polysaccharide moiety it could be envisioned that the resultant of phosphorylase addition to a membrane suspension would be phosphorolytic activity. It was found that membrane does not serve as a substrate for phosphorylase in a reaction which concomitantly produces glucose-1-phosphate since when the phosphorylase determination is conducted with membrane substituting for the omitted glycogen there is no evidence of reaction. Phosphorylase which is first exposed to membrane and then to an excess of glycogen exhibits diminished enzymic activity and this diminution varies directly with the membrane concentration and the duration of the initial exposure. Typical data are presented in Table II.

These data are compatible with a non-competitive inhibition of phosphorylase activity by membrane and argue against membrane serving as a substrate in a reaction which does not produce glucose-1-phosphate.

Curves for the optical rotation as a function of wave length,

TABLE II

EFFECT OF CONCENTRATION AND OF DURATION OF EXPOSURE OF MEMBRANE UPON PHOSPHORYLASE ACTIVITY

A 250 μg aliquot of phosphorylase was added to 6.0 ml of membrane suspension. Aliquots of 0.5 ml were removed at the times specified and introduced immediately into 2.0 ml of the medium for phosphorylase determination.

OPTICAL DENSITY OF MEMBRANE PREPARATION	TIME OF REMOVAL OF ALIQUOT	PERCENT INITIAL ACTIVITY
0.100	30 seconds	100
	10 minutes	100
	20 minutes	84
0.173	30 seconds	92
	10 minutes	81
	20 minutes	70
0.382	30 seconds	74
	10 minutes	72
	20 minutes	69

from 320 mμ to 195 mμ, were obtained for the enzymes phosphoglucomutase, phosphorylase, pyruvic kinase, and hexokinase and also for isolated kidney cell membrane. Each of the enzyme curves exhibited the characteristic negative Cotton effect which displays a trough at 233 mμ and a peak in the region of 198 mμ. The curves for membrane exhibited a maximum at 202 mμ and a minimum at 197 mμ. When phosphoglucomutase or hexokinase was added to membrane the resultant optical rotatory dispersion curves were identical with the theoretical summation curves however, the curves obtained when phosphorylase or pyruvic kinase was added to membrane differed from the theoretical. Figure 1 shows a typical optical

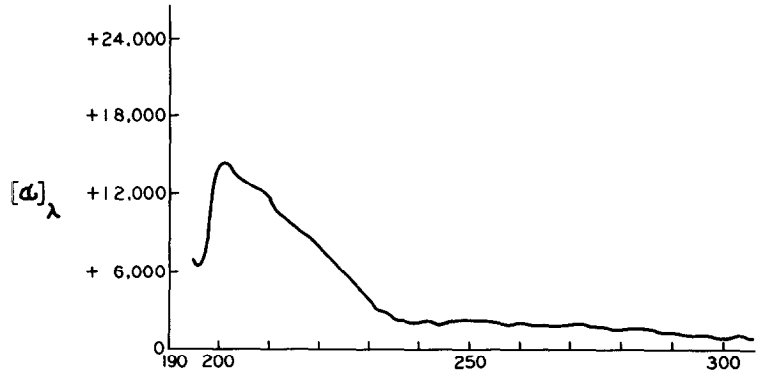

Fig. 1. Optical rotatory dispersion curve obtained using an isolated kidney cell membrane preparation having an optical density of 1.0 at 210 mμ, pH 7.48.

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rotatory dispersion curve for isolated kidney cell membrane. Figures 2 and 3 show the theoretical summation curves and the actual curves obtained when phosphorylase or pyruvic kinase, respectively, was added to membrane. Interaction between phosphorylase and membrane is reflected by an optical rotatory dispersion curve which exhibits an enhanced amplitude of its Cotton effect and a shift of the cross-over point toward shorter wave lengths. These data are compatible with an increase in secondary structure. The interaction of pyruvic kinase and membrane results in a curve in which the amplitude of its Cotton effect is diminished and it is suggested that there has been a decrease of helical structure. It should be noted that the activity of each of these two enzymes is inhibited by the presence of membrane. Data for phosphorylase have been presented above. The activity of pyruvic kinase was inhibited 4% by a membrane preparation having an optical density of 0.068 and 7.5% by one of optical density 0.230. In contrast, there was no evidence of

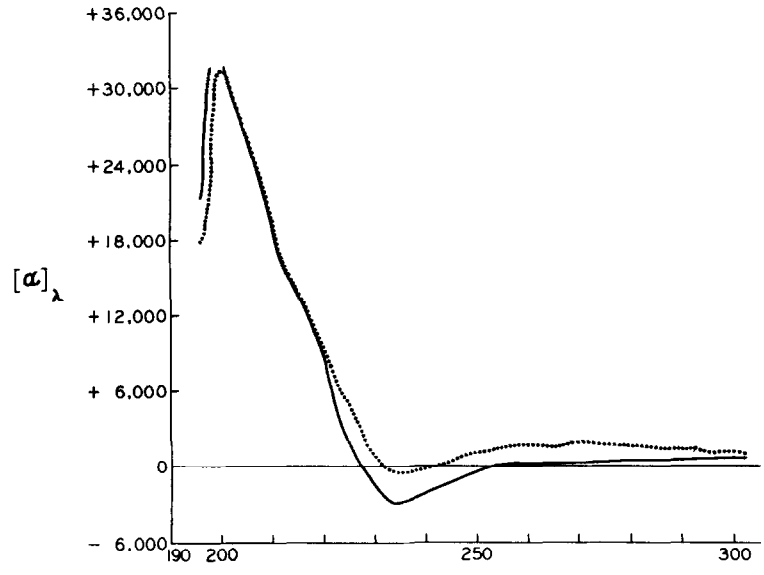

Fig. 2. Theoretical (.....) and actual (_____) optical rotatory dispersion curves obtained when phosphorylase, final concentration 40 µg/ml, is added to an isolated cell membrane preparation, optical density 1.0 at 210 mµ, pH 7.48.

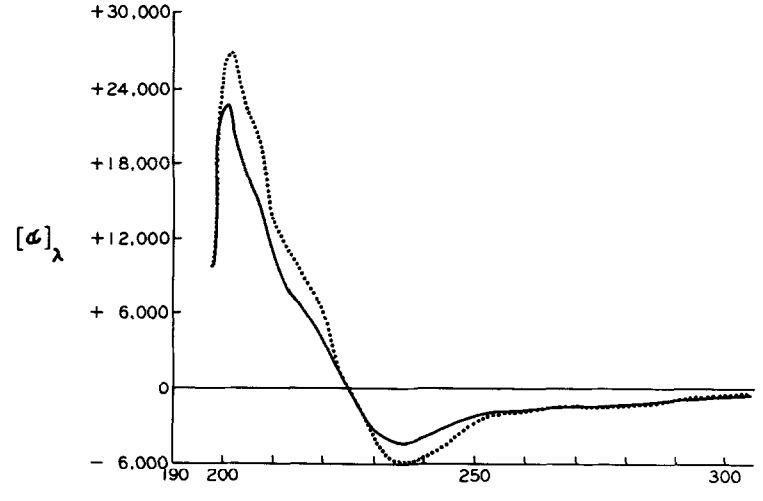

Fig. 3. Theoretical (.....) and actual (_____) optical rotatory dispersion curves obtained when pyruvic kinase, final concentration 40 µg/ml, is added to an isolated cell membrane preparation, optical density 0.7 at 210 mµ, pH 7.48.

similar conformational changes resulting from membrane interaction with either phosphoglucomutase or hexokinase. We have found the enzymic activity of phosphoglucomutase to be enhanced 6% by a membrane preparation having an optical density of 0.125 and enhanced 31% by a preparation having an optical density of 0.220 and it has been reported that hexokinase activity is enhanced when this enzyme is bound to mitochondrial membrane (Siekevitz, 1962).

Our results provide evidence for an interaction between kidney cell membrane and each of three phosphate-transferring enzymes which have a similar substrate requirement. The possible significance of such an interaction with respect to kidney function is being investigated.

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