PHOSPHORYLATION OF GLYCERALDEHYDE, GLYCERIC ACID AND DIHYDROXYACETONE BY KIDNEY EXTRACTS

by

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The phosphorylation of glucose, fructose and glycerol by cell-free extracts of kidney was first demonstrated by KALCKAR[1]. COLOWICK, WELCH, AND CORI[2] later found that adenylic acid, diphosphopyridine nucleotide, Mg^{++} and an oxidizable substrate were essential components of this phosphorylating system, in addition to inorganic phosphate. Evidence for the presence of a similar system in extracts of heart, brain and liver was obtained by COLOWICK, KALCKAR, AND CORI[3]. In the present experiments, it is shown that three additional compounds of physiological interest can be phosphorylated by dialyzed kidney extracts.

EXPERIMENTAL

The methods used were essentially as described by COLOWICK, WELCH, AND CORI[2]. Kidney cortex from young rabbits was homogenized at 0° with 1.5 volumes of 0.1 *M* potassium phosphate buffer, p_H 7.7, in a stainless steel homogenizer of the POTTER-ELVEHJEM type. The resulting paste was centrifuged for 5 minutes at 3000 r.p.m. at 2°. The cloudy, cell-free supernatant fluid was dialyzed for 6 hours against 0.05 *M* potassium phosphate buffer, p_H 7.7 at 2°. After dialysis, 3 μM of $MgCl_2$, 1 μM of adenosinetriphosphate, 100 μM of NaF and 0.5 μM of DPN were added in that order per ml of extract.

2 ml portions of this mixture (equivalent to 0.6 g of kidney cortex) with 0.3 ml of additions described in the Tables were shaken in WARBURG vessels at 37° in an atmosphere of O_2. The O_2 consumption was calculated for the entire incubation period, a correction being applied to include the O_2 consumed during the equilibration period of 8 minutes.

After incubation, the samples were deproteinized with trichloroacetic acid and analyzed for inorganic orthophosphate, additional orthophosphate liberated after 3 minutes in 0.1 *N* NaOH at 100° and after 10 and 60 in 1 *N* H_2SO_4 at 100°. Inorganic pyrophosphate was determined by the cadmium method of COHN AND KOLTHOFF[4].

Table I shows that some inorganic orthophosphate disappeared without the addition

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of a phosphate acceptor. An uptake of phosphate greater than that in the control was observed when glucose, fructose, glycerol, glyceric acid and dihydroxyacetone were added. Negative results were obtained with serine, ethanolamine, D-ribose, propanediol, glycol, glycolic acid, choline, ascorbic acid and D-gluconic acid.

TABLE I

EFFECT OF VARIOUS SUBSTANCES ON PHOSPHATE ESTERIFICATION BY KIDNEY EXTRACTS

The incubation mixture contained 2.0 ml of kidney extract with the additions given in the text, 40 micromoles of glutamate, 4 micromoles of succinate and 100 micromoles of the test substance in a final volume of 2.3 ml. Values are expressed in micromoles.

Experiment number	Substance added	Values after 60 min. incubation		
		O_2 uptake	Inorganic orthophosphate	Uptake of inorganic orthophosphate
I			60.0*	
	None	70.0	50.6	9.4
	Glucose	61.0	11.0	49.0
	Fructose	60.5	11.2	48.8
	Glycerol	63.5	13.9	46.1
	DL-Glyceric acid	67.6	31.0	29.0
	Dihydroxyacetone	84.5	27.5	32.5
	DL-Glyceraldehyde	33.1	48.0	12.0
	Serine	68.8	57.0	3.0
	Ethanolamine	67.6	47.6	12.4
	D-Ribose	73.0	50.4	9.6
II			79.2*	
	None	32.5	63.0	16.2
	Glucose	22.1	19.2	60.0
	1,2-Propanediol	36.3	60.1	19.1
	1,3-Propanediol	36.5	58.9	20.3
	Glycol	34.5	59.2	20.0
	Glycolic acid	27.0	61.6	17.6
	Choline	34.1	59.2	20.0
	Ascorbic acid	36.0	64.0	15.2
	D-Gluconic acid	28.0	66.2	13.0

* Value at 0 minutes

DL-glyceraldehyde was also inactive as phosphate acceptor when 100 μM were added to the system. In this concentration it caused a decrease in O_2 consumption of more than 50%, and differed in this respect from the other substances tested. Accordingly, smaller amounts of glyceraldehyde were added in the experiment in Table II in order to reduce its inhibitory effect on respiration. Under these conditions, the uptake of inorganic phosphate was significantly greater than in the control.

Some information about the nature of the phosphate esters which accumulate in this system in the presence of fluoride can be gained from an examination of Table III. It may be seen that there was an uptake of 20.4 μM of inorganic orthophosphate when no phosphate acceptor was added. The product formed while stable to alkali, was completely hydrolyzed in 10 minutes in 1 N H_2SO_4 at 100°; it was isolated as the crystalline cadmium salt according to the procedure of COHN AND KOLTHOFF[4] and

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TABLE II

EFFECT OF DL-GLYCERALDEHYDE ON PHOSPHATE ESTERIFICATION BY KIDNEY EXTRACTS

The incubation mixtures were the same as in Table I. In vessel 3, 15 micromoles of glyceraldehyde were mixed with the other reactants at 0 minutes and 15 micromoles were tipped in from the sidearm after 20 minutes incubation. The incubation time was 60 minutes. Values are expressed in micromoles.

Vessel number	Amount of DL-glyceraldehyde added	O_2 uptake	Uptake of inorganic orthophosphate
1	100	30.0	22.4
2	30	38.6	28.6
3	15 + 15	59.8	33.4
4	0	70.0	19.6

TABLE III

HYDROLYSIS OF PHOSPHATE ESTERS BY ALKALI AND ACID

The incubation mixtures were the same as in Table I. 100 μM of the test substances were added, except DL-glyceraldehyde. In the latter case 15 μM were mixed with the other reactants at 0 minutes and 15 μM were tipped in from the side arm after 20 minutes of incubation. The incubation time was 60 minutes. Values are expressed in micromoles and in per cent of the inorganic phosphate taken up which was hydrolyzed.

Substance added	O_2 uptake	Inorganic orthophosphate							Inorganic** pyrophosphate
		Before hydrolysis	After 3 min in 0.1 N NaOH		After 10 min in N H_2SO_4		After 60 min in N H_2SO_4		
	μM	μM	μM	%	μM	%	μM	%	
		89.9*							
None	69.0	69.5	74.6	25.0	89.9	100.0	94.3	121.0	20.3
Glucose	75.7	13.3	27.6	18.7	42.6	38.2	63.9	66.0	
Fructose	79.0	8.0	17.7	11.8	44.4	44.4	71.2	77.2	
Glycerol	80.5	36.1	40.7	8.5	49.5	24.9	54.0	33.3	12.0
DL-Glyceric acid	72.0	47.6	51.5	9.2	63.5	37.6	70.6	54.4	11.0
Dihydroxyacetone	64.0	57.0	65.8	26.8	69.9	39.2	79.9	69.6	
DL-Glyceraldehyde	74.0	55.1	62.5	21.3	79.9	71.2	87.5	93.1	15.2

* Value at 0 minutes

** Precipitated as the cadmium salt and calculated as orthophosphate

identified as inorganic pyrophosphate*. Inorganic pyrophosphate was also formed when phosphate acceptors were added, as shown in the last column of Table III. For this reason, the values for the 10 minute hydrolysis period in 1 N H_2SO_4 are not very useful for the purpose of identification of the other phosphate esters present. The increment in per cent hydrolysis between 10 and 60 minutes is free of this objection, since it does not include inorganic pyrophosphate. On this basis, the products formed with glycerol and glyceric acid as phosphate acceptors (increments of 8 and 17%, respectively) were more difficult to hydrolyze in acid than those formed from glucose, fructose and dihydroxyacetone (increments of 28, 33 and 30%, respectively). This is consistent with

* The formation of inorganic pyrophosphate was originally detected in experiments with liver homogenates[5]; examples are given in a preceding paper[6]. The mechanism of formation of this compound has been explained by KORNBERG[7].

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the idea that glycerol was converted to glycerophosphate, glyceric acid to phosphoglycerate, and the other 3 compounds to hexose-diphosphate*. Dihydroxyacetone, in particular, would yield dihydroxyacetone phosphate as the primary product, followed by isomerization and condensation to fructose diphosphate through the actions of triose isomerase and aldolase. If the primary product remained unchanged, it would be hydrolyzed completely by exposure to 0.1 *N* NaOH at 100°. Table III shows that the phosphorylation products which accumulated with the two trioses as phosphate acceptors were not alkali-labile.

Glyceraldehyde can be oxidized to glyceric acid by glyceraldehyde-3-phosphate dehydrogenase, but the rate of this reaction is very slow. If oxidation of glyceraldehyde preceded phosphorylation, one would expect the acid hydrolysis curves for the products of glyceric acid and glyceraldehyde phosphorylation to be similar. Table III shows, however, that this is not the case, and that the product of glyceraldehyde phosphorylation is almost completely hydrolyzed in 60 minutes in 1 *N* H_2SO_4 at 100°. This suggests the formation of fructose-1-phosphate and sorbose-1-phosphate through condensation of dihydroxyacetone phosphate with the D- and L-components of glyceraldehyde[8]. On this view, the primary products of glyceraldehyde phosphorylation would be D-glyceraldehyde-3-phosphate which is in enzymatic equilibrium with dihydroxyacetone phosphate. Both trioses can be converted to glucose when incubated with liver slices in the absence of fluoride. The pathway for this conversion, following the initial phosphorylation, is described in a preceding paper[6].

SUMMARY

Glyceric acid, glyceraldehyde and dihydroxyacetone can be phosphorylated in a dialyzed kidney homogenate supplemented by an oxidizable substrate and catalytic amounts of adenosinetriphosphate and diphosphopyridine nucleotide. This was shown by measurement of the uptake of inorganic phosphate and characterization of the products formed on the basis of acid and alkali hydrolysis. The following other substances did not lead to a phosphate uptake which was significantly greater than that of the control: serine, ethanolamine, ribose, propanediol, glycol, glycolic acid, choline, ascorbic acid and gluconic acid. The inorganic phosphate which disappeared in the absence of phosphate acceptor was largely converted to inorganic pyrophosphate which was isolated as the cadmium salt.

RÉSUMÉ

L'acide glycérique, le glycéraldéhyde et la dioxyacétone peuvent être phosphorylés dans un homogénat de rein dialysé complété par un substrat oxydable et de l'adénosine triphosphate et du diphosphopyridine-nucléotide en quantité catalytique. Nous avons pu démontrer ce fait en mesurant la quantité de phosphate inorganique absorbé et en caractérisant les produits formés par hydrolyse acide et alcaline. Les substances suivantes n'ont pas donné lieu à une absorption de phosphate considérablement plus importante que celle de l'essai à blanc: sérine, éthanolamine, ribose, propanediol, glycol, acide glycolique, choline, acide ascorbique et acide gluconique. Le phosphate inorganique disparu en l'absence d'un accepteur de phosphate était transformé en grande partie en pyrophosphate inorganique qui a été isolé comme sel de cadmium.

ZUSAMMENFASSUNG

Glycerinsäure, Glycerinaldehyd und Dioxyaceton können in einem dialysierten Nierenhomogenat, welches mit einem oxydierbaren Substrat und katalytischen Mengen von Adenosintriphosphat und Diphosphopyridin-nukleotid ergänzt ist, phosphoryliert werden. Dies wurde durch Messung der

* It has been shown previously that fructose diphosphate accumulates in this system when glucose is the phosphate acceptor and fluoride is added[2,3].

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Aufnahme von anorganischem Phosphat und durch Charakterisierung der Reaktionsprodukte mit Hilfe von saurer und alkalischer Hydrolyse nachgewiesen. Die folgenden anderen Substanzen führten nicht zu einer Phosphataufnahme, welche bedeutend grösser war, als die des Blindversuches: Serin, Äthanolamin, Ribose, Propandiol, Glykol, Glykolsäure, Cholin, Ascorbinsäure und Glukonsäure. Das anorganische Phosphat, welches in Abwesenheit eines Phosphatacceptors verschwand, wurde grösstenteils in anorganisches Pyrophosphat verwandelt, welches als Cadmiumsalz isoliert wurde.

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