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LIVER URIDINE PHOSPHORYLASE

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Some animal tissues contain enzymes which split off the base from nucleosides. Klein [1] studied their action on purine and pyrimidine nucleosides and called them nucleosidases. He observed that their activity disappears after dialysis and reappears in the presence of phosphate or arseniate.

The mechanism of action one of these enzymes was elucidated by Kalckar [2]. In the course of studies on the estimation of nucleosides [3] he rediscovered the necessity of phosphate and was able to prove that ribose-1-phosphate was formed in the reaction. The latter substance is very acid-labile, so that the phosphate estimations had to be carried out with the method of Lowry and López [4] which avoids the use of strong acid.

Kalckar found that with a rat liver enzyme and inosine (hypoxanthine riboside) or guanosine as substrates, the following reversible reactions took place:

Hypoxanthine riboside + phosphate $\rightleftarrows$ hypoxanthine + ribose-1-phosphate Guanosine + phosphate $\rightleftarrows$ guanine + ribose-1-phosphate.

Due to the similarity of the reaction with that catalyzed by polysaccharide phosphorylase the enzyme was named nucleoside phosphorylase. The ribose-1-phosphate formed appears to be the furanoside since it was found that synthetic ribopyranose-1-phosphate was not used in the reaction going from right to left, that is in the synthesis of nucleosides.

Kalckar was unable to detect any action of the enzyme on adenosine, xanthosine or pyrimidine ribosides. However, the experiments of Schlenk and Waldvogel [5] show that ribose-phosphate is formed also from adenosine.

Similar reactions have been found to occur with desoxyribose-nucleosides. Manson and Lampen [6] obtained an enzyme from thymus which catalyzed the formation of desoxyribose-phosphate from hypoxanthine desoxyriboside and inorganic phosphate. The ester formed was beliefed. to be desoxyribose-5-phosphate. They also detected a similar enzyme in bone marrow and kidney which acts on desoxypyrimidine-nucleosides, specially thymidine [7]. Similar results have been obtained by Wajzer [8].

The formation of desoxyribose-1-phosphate from guanine-desoxyriboside was detected by Friedkin, Kalckar an Hoff-Jorgensen [9]. The reaction was found to be reversible and the nucleoside could be synthesided from hypoxanthine and desoxyribose-1-phosphate [2] The latter substance was found to be even more acid-labile than ribose-1-phosphate.

Crude extracts of liver and other tissues catalyze the formation of ribose-5-phosphate from the 1-isomer. This reaction is similar to the transformation of the glucose-1-phosphate into the 6-phosphate which requires glucose 1,6-diphosphate as coenzyne [10]. It is therefore likely that the coenzyme of phosphoribomutase is ribose-1,5- disphosphate [11]. The existence of a similar enzyme for the desoxyribose-phosphate has been postulated by Manson and Lampen [6]

Schlenk and Waldvogel [5] have observed that when guanosine or adenosine is incubated with liver extracts and phosphate, the ribose disappears and is replaced by an acid stable phosphoric ester. They were able to prove [12] that about half the ribose-phosphate was transformed into hexose-6-phosphate. The same transformation ocurred starting with ribose-5-phosphate but not with free ribose or ribose-3-phosphate.

A similar observation had been made by Dische [13] several years before. In human red blood cells the ribose of adenosine disappeared. He suggested that the pentose was transformed into triose- phosphate and a two carbon compound. Using a bacterial enzyme Racker [14] detected the formation of triose-phosphate from ribose-5-phosphate. With muscle aldolase he observed a condensation of triose-phosphate with glycolic aldeyde to a pentose-phosphate. However, the product was not ribose-phosphate nor was there any evidence of its transformation into this substance.

The synthesis of inosinic acid has been reported by Wajzer and Baron [15-16] by incubation of inosine, ribose-3-phosphate and a liver enzyme. The inosinic acid was estimated by its activating action of polysaccharide phosphorylase.

Interest in the metabolism of uridine was aroused in this laboratory as a consequence of the isolation of uridine-diphosphate-glucose [17] which acts as a coenzyme in the galactose-1-phosphate → glucose-1-phosphate transformation. It was found that rat liver contains an enzyme which removes uracil from ribose, which is activated by phosphate and which leads to the formation of ribose-phosphate. The enzyme therefore catalyzes the phosphorolysis of uridine. In a note Paege and Schlenk [18] have recently reported the presence of a similar enzyme in bacteria.

Experimental

Preparation of the enzyme. — Rats were killed and the liver was immediately homogeneized in two volumes of water. After standing two or three hours in the ice-box, the homogenate was centrifuged at 3.000 r.p.m. The supernatant was then dialyzed against distilled water for 15 to 20 hours. Dialysis for a longer time did not inactivate the enzyme. On storage for several days at −5ºC inactive protein precipitated and no appreciable loss of activity was observed. In some experiments the extract of acetone dried liver as described by Schlenk and Waldvogel [5] was used, and in others the extract prepared according to Kalckar [3] taking the fraction which precipitates between 0.4 and 0.6 saturated ammonium sulphate followed by dialysis.

TABLE I

Action of the liver extract on different substrates. Results in micromoles of ribose-5-phosphate

SUBSTRATE	Hours of incubation			
	1	2	3	5
Uridine	0.14	0.25	0.29	0.29
Uridine-3'-phosphate	0.12	0.17	0.21	0.24
Uridine-5'-phosphate	—	—	0.29	—
Cytosine	0	0	0	—
Cytosine-3'-phosphate	0	0	0	—

TABLE II

The action of phosphate and magnesium. Procedure as described in text. Substrate: uridine. Results in micromoles of ribose-5-phosphate

Hours of incubation	No phosphate No magnesium	No phosphate With magnesium	With phosphate With magnesium
1	0	0.02	0.15
2	0.03	0.02	0.16

TABLE III

The action of magnesium ion concentration. Procedure as described in text. Incubation during 2 hours at 37°C. Results in micromoles of ribose-5-phosphate

Molar concentration of Mg	0	.0013	.0025	.005	.01	0.02	0.04
Uridine	.09	—	0.12	0.15	0.20	0.22	0.22
Uridine-3'-phosphate	.04	0.07	0.10	0.11	0.15	0.18	—

TABLE IV

pH optimum. Results in micromoles of ribose-5-phosphate

SUBSTRATE	pH			
	6.6	7.0	7.4	8.0
Uridine	0.06	0.08	0.18	0.17
Urdiylic acid.	0.11	0.12	0.13	0.11

Substrates. — Uridine, uridine-3'-phosphate, cytosine and cytosine-3'-phosphate were prepared according to Loring *et al* [19] or were commercial samples (Schwarz). Uridine-5'-phosphate was prepared from uridine-diphosphate-glucose [17].

Estimation of the enzyme. — The ribose of pyrimidin-ribosides gives no colour with the methods used for pentose estimation. Therefore, the liberation of ribose or ribose-phosphate can be easily detected.

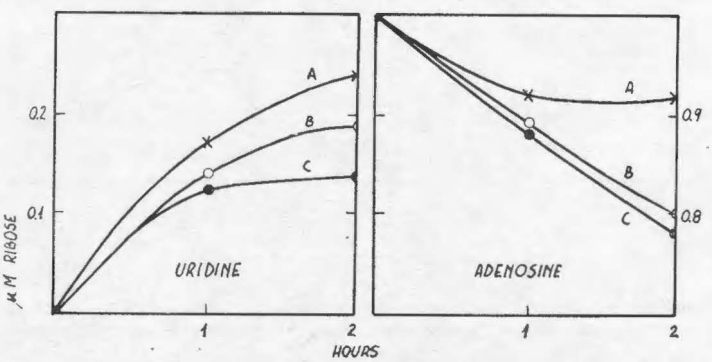

FIGURE 1. — The action of different enzyme preparations on uridine and adenosine.

x Liver extract dialyzed 20 hours.

• Fraction precipitating between 0.4 — 0.6 saturation with ammonium sulphate following the procedure of Kalckar (3).

o Extract of acetone dried liver according to Schlenk and Waldvogel (5).

The enzymatic system was made up as follows: 1 micromole of substrate, plus 0.1 ml of 0.06 M phosphate buffer of pH 7.4 plus 0.1 ml of 0.08 M magnesium chloride and 0.1 ml of enzyme solution. Total volume, 0.3 to 0.4 ml. Incubation was carried out at 37°C in the presence of toluene.

The reaction was interrupted at the desired time by addition of one volume of 2 N HCl; after centrifugation an aliquot of the supernatant, usually half, was taken. Water was then added to make 3 ml and the ribose was estimated following the method described by Mejbaum [20], heating 30 minutes. In some cases the coloured compound was extracted with amyl alcohol. Ribose or xylose were used as standards and the colorimetric values were calculated as ribose-5-phosphate following the indications of Albaum and Umbreit [21].

RESULTS

Action on different substrates. — As shown in table I, the liberation of ribose could be detected with uridine or its phosphates, but not with cytosine. With the latter the addition of heated liver extracts was tested with negative results. The uridine-phosphates are probably transformed into uridine of phosphatase action.

Accurate results of the activity of uridine-phosphorylase were not obtained because the system contains enzymes which catalyze the disappearance of ribose-phosphate. This can be observed in fig. 1. When adenosine is the substrate the ribose moiety is directly estimated with Mejbaum's procedure. By incubation with the enzyme mixture the values decrease due to the further transformation of the ribose-phosphate liberated by the phosphorylase. With uridine the ribose moiety is not estimated until it is transformed into ribose-phosphate. The accumulation of ribose-phosphate from uridine depends on the relative activity of two reactions: the uridine-phosphorylase which forms ribose-phosphate and the other enzymes which destroy it. The results using the enzyme preparations of Kalckar and Schlenk and Waldvogel are also shown in fig. 1.

Conditions for maximum activity. — Results appearing in table II show that practically no enzymatic activity was detected in the absence of either phosphate or magnesium ions. The effect of the latter was more manifest after a thorough dialysis. The optimum concentration of magnesium is 0.02 to 0.04 M or higher (table III).

TABLE V

The formation of ribose esters. Procedure described in text. Amount doubled. Substrate: uridine. Ribose esters represent the part precipitated by the Somogyi deproteinizing procedure.

Hours of incubation	Free ribose μ moles	Ribose esters μ moles
1	0.04	0.30
2	0.04	0.50
3	0.06	0.53

TABLE VI

Liberation of uracil. Substrate: 2 micromoles of uridine. Uracil estimated by absorbancy at 290 mμ in 0.01 NaOH after deproteinizing with trichloracetic acid.

Hours of incubation	Ribose esters formed	Uracil liberated
2	0.40	0.46
3	0.46	0.54

The pH optimum was about 7.4 both when the substrate was uridine or uridylic acid (table IV).

The formation of ribose ester. — It is known that carbohydrate phosphates are precipitated in the deproteinization procedure described by Somogyi [22]. Use of this fact was made in order to find out whether the ribose liberated from uridine was free or esterified. The ribose left in the supernatant after zinc sulphate barium hydroxide treatment was substracted from the amount found directly. This value was considered to represent esterified ribose.

As shown in table V, practically all the ribose liberated by the enzyme is in the bound form.

The liberation of uracil. — The absorption spectra of uracil and uridine published by Ploeser and Loring [23] show that in 0.01 N sodium hydroxide the molar absorbency index (molar extinction coefficient) at 290 mμ for uracil is 5 500 and practically zero for uridine. It is thus possible to estimate these two substances in a mixture. As shown in table VI, the amount of uracil liberated is slightly higher than the amount of ribose esters formed.

Discussion

The reactions catalyzed by the enzyme mixture can be formulated as follows:

I Uridine + phosphate $\rightleftarrows$ uracil + ribose-1-phosphate.

II Ribose-1-phosphate $\rightleftarrows$ ribose-5-phosphate.

III Ribose-5-phosphate $\rightleftarrows$ unidentified products.

That reaction I takes place is proved by the liberation of uracil, by the indispensability of phosphate for the reaction and by the formation of ribose ester. Reactions II and III also take place with the extract used because starting with adenosine there occurs a disappearance of ribose, the mechanism of which has been studied by other workers.

The necessity of magnesium ions has not been mentioned in the work of Kalckar on guanosine-phosphorylase. It is likely that magnesium is necessary for reaction II and not for the phosphorylase (reaction I). The activation observed would be due to the fact that reaction I is reversible and would not take place if the ribose-1-phosphate is not removed from the reaction mixture by the phosphoribomutase (reaction II).

The activity of nucleoside phosphorylase which was found by Kalckar to be limited to the guanine and hypoxanthine ribosides, appears to be more general. Thus activity on adenosine was found by Schlenk and coworkers and activity on uridine is described in this paper. It is likely that the discrepancies are due to the methods of estimation and on the position of the equilibrium in these reactions.

Another point which remains to be settled is whether there is only one phosphorylase which acts on several ribosides or whether each nucleoside needs a specific enzyme.

Summary

Rat liver extracts were found to contain an enzyme which in the presence of inorganic phosphate transforms uridine into uracil and ribose phosphate. The process was found to be accelerated by magnesium ions.

Uridylic acid was also transformed whereas cytosine and cytidylic acid were not.

Resumen

En el extracto de hígado de rata se demostró la presencia de una fosforilasa que actúa sobre la uridina y los ácidos uridílicos originando, en presencia de fosfato y magnesio, uracilo y ésteres de ribosa.

La citosina y el ácido citidílico no son atacados.

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