ON THE SYNTHESIS OF NUCLEOTIDES BY NUCLEOSIDE PHOSPHOTRANSFERASES*

by

GEORGE BRAWERMAN** AND ERWIN CHARGAFF

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York (U.S.A.)

INTRODUCTION

Preliminary observations[1,2] have led to the discovery of a group of enzymes capable of transferring organically esterified phosphoric acid to nucleosides, thereby effecting the synthesis of nucleotides by low-energy phosphate transfer. For this group of enzymes we propose the general designation "nucleoside phosphotransferases".

These systems appear to be the first examples of agents capable of bringing about the formation of all the nucleotides existing in the cell. This is in contrast to the kinase specific for adenosine[3,4] and to the ribose-1,5-diphosphate system[5,6] which seems to operate only in the case of adenylic and inosinic acids.

The work of AXELROD on the transferase activity of certain phosphatase preparations[7] had shown that phosphate transfer reactions can occur in the absence of high energy donors, although the biological significance of the reactions studied was not apparent. Several authors have subsequently studied the phosphorylation of simple alcohols and sugars by such transfer enzymes[8–10]. In all cases very high concentrations of acceptor had to be used to allow the reaction to proceed to a measurable extent.

The transfer enzymes described in this paper effect the synthesis of mononucleotides. The reaction systems involved utilize low-energy phosphates as donors, but differ from the above mentioned transferases by their ability to proceed to an appreciable extent with relatively low concentrations of acceptor. The reaction was first studied with a preparation from commercial malt diastase that could form only small amounts of nucleotide together with large amounts of inorganic phosphate; but it was subsequently found that preparations with a considerably greater synthetic activity could be obtained from germinating wheat. The enzyme was able to phosphorylate all nucleosides tested, and this only on the 5 position of the sugar moiety; it showed in addition an interesting donor specificity. Subsequently, enzymes were found in various tissues that exhibited different specificities with respect to donors, acceptors, and nucleotide isomers synthesized. The apparent specificity of these enzymes towards nucleosides justifies the designation proposed above.

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On the basis of these specificity differences, the nucleoside phosphotransferases can at present be grouped into three classes which are represented in this paper by the enzymes from malt, rat liver, and human prostate.

MATERIALS AND ANALYTICAL METHODS

Sodium monophenylphosphate was prepared according to ASAKAWA[11], ribose-5-phosphate (Ba salt) was made from 5'-adenylic acid by a method described by KHYM AND COHN[12]. The deoxyribosides of hypoxanthine, adenine and guanine, the deoxyribonucleotides of adenine and cytosine, and 5'-ribocytidylic acid were kindly furnished by Dr. WALDO E. COHN, Oak Ridge National Laboratory; uracil deoxyriboside by Prof. A. R. TODD, University of Cambridge; and thymine deoxyriboside by Dr. M. E. HODES of this laboratory. The other compounds used were commercial products.

The malt enzyme has been described in a previous paper[13]; Preparation II was used. The wheat extract was obtained from 10 days old wheat shoots (10 to 15 cm high), separated from the seeds frozen and treated in a high-speed mixer with ice-cold distilled water. After centrifugation and dialysis against distilled water, the material was lyophilized. The liver extract was obtained by treating fresh or frozen rat livers in a high-speed mixer with distilled water, centrifuging the mixture at 500 × *g* for 30 minutes, dialyzing the supernatant fluid and lyophilizing. The prostate phosphatase was prepared according to LORING[14]. The venom from rattlesnake (*Crotalus adamanteus*), used as 5'-nucleotidase[15], was purchased from Ross Allen's Reptile Institute, Silver Springs, Florida. The *b* nucleotidase[16] was kindly furnished by Dr. N. O. KAPLAN, Johns Hopkins University, Baltimore.

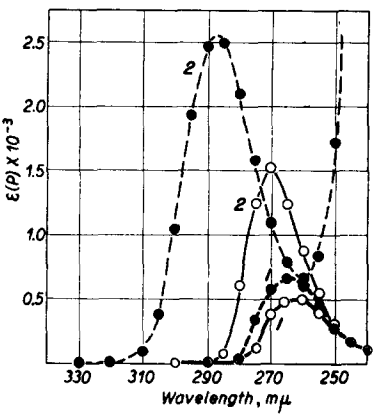

Fig. 1. Ultraviolet absorption of phenylphosphate and phenol. 1, Phenylphosphate; 2, phenylphosphate treated with prostate phosphatase; ● in 0.1 *M* NaOH; ○ in 0.1 *M* acetate buffer, pH 5.2. ε(P) represents the atomic extinction coefficient with respect to phosphorus[17].

Phenol was determined by spectrophotometry in the ultraviolet. The values of $E_{295}-E_{320}$ and of $E_{290}-E_{320}$ used in this work for a 10^{-3} *M* solution of phenol in 0.1 *N* NaOH were 1.92 and 2.45, respectively. The molar extinction curves in Fig. 1 show that the hydrolysis of phenylphosphate results in a large increase in ultraviolet absorption at both pH 5 and 11. This observation could form the basis for a spectrophotometric assay of phosphatase activity.

Nucleosides and nucleotides were separated by paper chromatography with aqueous *iso*butyric acid buffered with ammonium *iso*butyrate[18]. The U.V. absorption data given by VOLKIN AND COHN[20] and the relative R_F values listed in Table I were used for their identification and determination. Most of the measurements were made in 0.1 *M* phosphate buffer, pH 7, except for the cytidine derivatives, where 0.1 *M* HCl was used.

Phosphate was determined by the method of KING[21].

EXPERIMENTAL

Transphosphorylation by the malt enzyme

Phosphorylation ratio. Since the phosphotransferase preparations contain phosphatase activity which will attack both the phosphate donor and the nucleotide formed, it is important to compare what may be called the synthetic and hydrolytic activities. It can be seen in Fig. 2 that the amount of nucleotide formed is, at the outset, proportional to that of inorganic phosphate produced. The relative rate of synthesis then falls off, and when a large proportion of the donor has been split the absolute amount of nucleotide present in the digest begins to decrease. The molar ratio of phosphate transferred to inorganic phosphate formed, which will be called here the "phosphorylation

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TABLE I

CHROMATOGRAPHIC BEHAVIOR OF DONORS, ACCEPTORS, AND REACTION PRODUCTS; RELATIVE R_F VALUES*

Substance	*Test substances*	*Nucleotides produced enzymically*
Adenine riboside	1.14	
Adenine deoxyriboside	1.15	
5'-Riboadenylic acid	0.74	0.74
3'-Riboadenylic acid**	0.85	0.86
Deoxyriboadenylic acid	0.86	0.87
Guanine riboside	0.71	
Guanine deoxyriboside	0.85	
5'-Riboguanylic acid		0.28
Deoxyriboguanylic acid	0.51	0.51
Hypoxanthine riboside	0.74	
Hypoxanthine deoxyriboside	0.87	
5'-Riboinosinic acid	0.41	0.42
Deoxyriboinosinic acid		0.55
Cytosine riboside	0.95	
Cytosine deoxyriboside	1.07	
5'-Ribocytidylic acid	0.58	0.60
3'-Ribocytidylic acid**	0.67	0.68
Deoxyribocytidylic acid	0.74	0.73
Uracil riboside	0.73	
Uracil deoxyriboside	0.88	
5'-Ribouridylic acid		0.41
Deoxyribouridylic acid		0.53
Thymine deoxyriboside	1.00	
5'-Thymidylic acid	0.63	0.66
3'-Thymidylic acid		0.66
Phenylphosphate	0.95	

* In *iso*butyric acid-ammonium *iso*butyrate, pH 3.6[18].
** The 2'-ribonucleotides migrate to the same positions as the 3'-isomers in this solvent.

TABLE II

EFFECT OF DONOR CONCENTRATION*

Phenyl-phosphate μmoles/ml		*Incubation time, hours*							
		1	*3*	*5*	*7*	*24*	*47*	*119*	*222*
40	P	6.6	15	18.5	25.5	39			
	Nc.	0.27	0.49	0.67	0.85	0.75			
	P.R.	0.041	0.033	0.036	0.033	0.019			
80	P		19		35	63	77.5		
	Nc.		0.67		1.21	1.87	1.64		
	P.R.		0.035		0.035	0.030	0.021		
200	P				39.5	87	124	162	190
	Nc.				1.34	3.34	4.23	4.38	4.21
	P.R.				0.034	0.038	0.034	0.027	0.022
300	P					87	131	179	212
	Nc.					3.18	4.64	4.64	4.39
	P.R.					0.037	0.035	0.026	0.021

* P denotes inorganic P and Nc. the nucleotides formed, both in μmoles per ml. Conditions of the experiment: 40 μmoles cytidine and 2 mg malt enzyme per ml of 0.1 *M* acetate buffer, pH 5.2; incubation at 30°. The concentration of phenol liberated from the substrate was sufficient to guarantee aseptic conditions.

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ratio" (P.R.)*, corresponds to the slope of the curve. The P.R. is a convenient measure of the synthesizing activity, but it will be significant only during the first stage of the reaction (up to about 50% inorganic phosphate formation), where it remains constant.

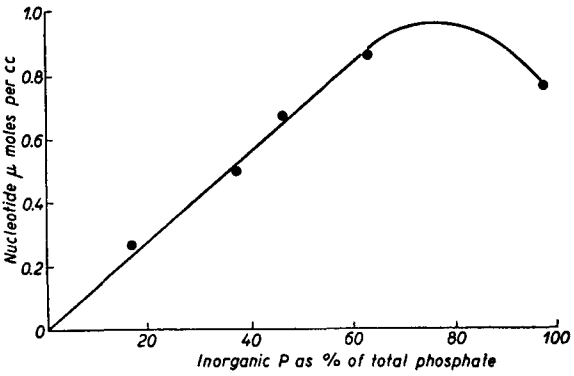

Fig. 2. Relation between phosphate transferred and inorganic phosphate formed. Conditions: 40 μ moles phenylphosphate, 40 μ moles cytidine, 2 mg malt enzyme per ml 0.1 *M* acetate buffer, pH 5.2; incubation at 30°.

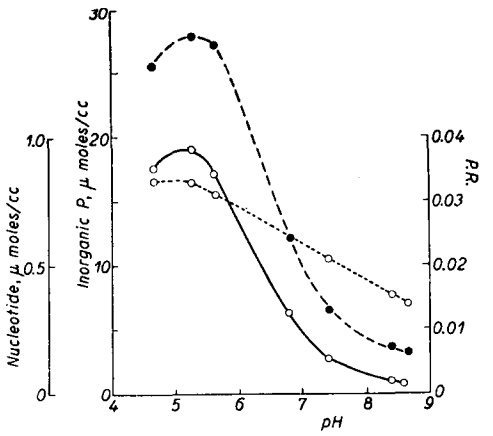

Fig. 3. pH-Activity curve for the malt phosphate transfer reaction. ● Inorganic phosphate formed; O (solid line) nucleotide formed; O (broken line) phosphorylation ratio. Conditions: 40 μ moles phenylphosphate, 40 μ moles cytidine, 2 mg malt enzyme per ml; mixed buffer 0.1 molar with respect to both tris(hydroxymethyl)aminomethane and sodium acetate; incubation 7 h at 30°.

pH-Activity curves. The curves for the phosphatase and phosphotransferase effects (Fig. 3) show a marked similarity, particularly below pH 7. The decrease in the P.R. values on the alkaline side indicates a higher relative phosphatase activity in that range.

Effect of donor and acceptor concentrations. An increase in the donor concentration results in higher yields of nucleotide, but leaves the P.R. unchanged (Table II). Table III shows that the P.R. varies with the concentration of acceptor, but not in a linear fashion. Nucleosides also produce an inhibition of the rate of phenylphosphate splitting. This effect is apparent only with the higher concentrations of nucleosides in Table III, but it occurs to a considerably greater extent with the more active wheat preparation.

TABLE III

EFFECT OF ACCEPTOR CONCENTRATION

Cytidine μmoles/ml	*Inorganic P formed μmoles/ml*	*Nucleotide formed μmoles/ml*	*P.R.*
5	77	0.84	0.011
10	77	1.42	0.018
20	76	2.10	0.028
50	72	3.10	0.043
100	69	3.80	0.055

Experimental conditions: 200 μ moles phenylphosphate and 2 mg malt enzyme per ml of 0.1 *M* acetate buffer, pH 5.2; incubation, 19 hours at 30°.

* This expression differs from the transfer ratio (ratio of phosphate transferred to total donor split) introduced by AXELROD[7].

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Effect of inorganic phosphate. Table IV shows that inorganic phosphate is not involved in the transfer reaction, since its addition does not increase the extent of phosphorylation. Its only effect is to inhibit the synthetic and hydrolytic reactions to the same extent.

TABLE IV

INFLUENCE OF INORGANIC PHOSPHATE

Phenylphosphate μmoles/ml	*Inorganic phosphate μmoles/ml*	*Phenol liberated μmoles/ml*	*Nucleotide formed μmoles/ml*	*P.R.*
200	—	43.5	1.60	0.037
200	100	30	1.14	0.038
—	100	—	0	—

Experimental conditions: 40 μmoles cytidine and 2 mg malt enzyme per ml of 0.1 *M* acetate buffer, pH 5.2; incubation, 7 hours at 30°.

Interpretation. The reactions just described can be represented by the following equations, with DP defined as the phosphate donor, D as the dephosphorylated donor, Ac as the acceptor, AcP as the phosphorylated acceptor, E as the enzyme, DPE as the enzyme-donor complex, and P as inorganic phosphate.

$$DP + E_1 \rightleftarrows DPE_1 \xrightarrow{Ac} AcP + D$$

$$DP + E_2 \rightleftarrows DPE_2 \xrightarrow{H_2O} P + D$$

The rates will be

$$\frac{d\,[AcP]}{dt} = k_1\,[DPE_1]\,[Ac]$$

$$\frac{d\,[P]}{dt} = k_2\,[DPE_2]$$

$$P.R. = \frac{d\,[AcP]}{d\,[P]} = k\,\frac{[DPE_1]}{[DPE_2]}\,[Ac]$$

According to this equation, the P.R. is dependent on $[DPE_1]/[DPE_2]$ and on the concentration of acceptor. $[DPE_1]$ and $[DPE_2]$ are dependent on the concentration of donor, but only below the saturation level of donor concentration. Below this level, the ratio $[DPE_1]/[DPE_2]$ would vary with the donor concentration, provided the Michaelis constants of the two enzymes are different. The concentrations used in this work were, however, close to the saturation level, and as should have been expected, the P.R. shows no such variation. The question whether the same enzyme is involved in both reactions cannot be settled by these results, since by making E_1 equal to E_2 a similar expression for the P.R. is obtained. A careful kinetic study of the behavior of $[DPE_1]/[DPE_2]$ with low concentrations of donor would throw more light on the matter, but this will have to be done with purified preparations of the enzyme.

The decrease in P.R. values observed in the later stages of the reaction is most probably due to the action of phosphatase on the newly formed nucleotide. This action was neglected in the present derivations, but becomes considerable as the protective effect due to the large amounts of phosphate donor fades away.

The non-linearity observed experimentally in the variation of the P.R. with acceptor concentration may be due to the saturation level of acceptor concentration being reached, unless a more complicated mechanism prevails.

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Specificity of the phosphotransferases

Acceptors. All nucleosides tested were phosphorylated. As shown in Table V, the prostate enzyme differentiates noticeably between ribo- and deoxyribosides, the liver enzyme between the uracil and cytosine ribosides.

TABLE V

ACCEPTOR SPECIFICITY OF THE NUCLEOSIDE PHOSPHOTRANSFERASES

Enzyme	*Experimental arrangement**	*Acceptor aglycone*	*P.R.*	
			Riboside	*Deoxyriboside*
Malt	1	Cytosine	0.035	0.043
		Uracil	0.026	0.035
		Thymine		0.033
		Hypoxanthine	0.022	0.036
		Adenine	(0.024)	(0.032)
		Guanine	(0.020)	(0.030)
	2	Guanine	0.004	0.006
		Cytosine	0.007	
	3	Adenine	0.016	0.021
		Cytosine	0.023	
Prostate	4	Adenine	0.052	
		Thymine		0.144
	5	Cytosine	0.046	0.151
		Uracil	0.049	
Liver	6	Cytosine	0.160	0.212
		Uracil	0.038	

* Unless stated otherwise, 200 μmoles phenylphosphate, 40 μmoles nucleoside per ml of 0.1 *M* acetate buffer, pH 5.2; incubation at 30°. Other experimental conditions were: 1. 2 mg enzyme per ml, incubation 30 h; inorganic P formed, 100 μmoles per ml; 2. 4 μmoles nucleoside and 3 mg enzyme per ml, incubation 45 h; inorganic P formed, 125 μmoles per ml; 3. 80 μmoles phenylphosphate and 2 mg enzyme per ml, 0.1 *M* formate buffer, pH 3.6, incubation 23 h; inorganic P formed, 40 μmoles per ml; 4. 0.05 mg enzyme per ml, incubation 25 h; inorganic P formed, 120 μmoles per ml; 5. 100 μmoles phenylphosphate and 0.015 mg enzyme per ml, incubation 3.5 h; inorganic P formed, 15 μmoles per ml; 6. 100 μmoles phenylphosphate and 5 mg enzyme per ml, incubation 3.5 h; inorganic P formed, 12 μmoles per ml.

The arrangement No. 2 was necessitated by the low solubility of the guanine nucleosides, and No. 3 served to minimize the action of the adenosine deaminase present in the malt enzyme[13]. The values in parentheses for adenosine and guanosine in arrangement No. 1 were computed from Experiments 2 and 3 with ribocytidine as the reference compound.

A few alcohols and sugars, namely ethanol, glycerol, D-ribose and D-fructose were tested for phosphorylation under conditions similar to those followed with the nucleosides (100 μ moles phenylphosphate and 40 μmoles acceptor per ml). The P.R. values obtained ranged between 0 and 0.03, but it is not certain that any phosphorylation occurred, since the standard deviations were of the order of 0.03 owing to the inaccuracy of the method available to measure transfer in this case (difference between phenol and inorganic P formed). In these experiments, the enzyme preparations from prostate and liver showing P.R. values up to 0.15 and 0.21 respectively with nucleosides (Table V),

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and from wheat seedlings, showing a P.R. value of 0.20 with ribonucleosides, were employed. It may be concluded that these nucleoside phosphotransferases were practically devoid of unspecific transfer activity.

Nucleotide isomers formed. Whereas the malt enzyme produces only the 5'-isomers and the nucleotides formed by the liver preparation consist to about 95% of the 5'-isomers, the prostate enzyme affords, in the ribose series, about equal amounts of 5'- and 3'-nucleotides together with smaller amounts of the 2'-isomer* (see Table VI). With thymidine about twice as much 5'- than 3'-thymidylic acid is produced.

TABLE VI

NUCLEOTIDE ISOMERS SYNTHESIZED BY THE DIFFERENT PHOSPHOTRANSFERASES*

Enzyme	*Acceptor*	*Nucleotides formed***		
		5'-isomer	*3'-isomer*	*2'-isomer*
Malt***	Ribocytidine	100	0	0
Liver§	Ribocytidine	95	5	
Prostate***	Ribocytidine	41	42	17
	Riboadenosine	35	53	12
	Thymidine	63	37	

* Experimental conditions: 200 μmoles phenylphosphate and 40 μmoles acceptor per ml; 0.1 *M* acetate buffer, pH 5.2; malt enzyme, 2 mg per ml (90 h); liver enzyme, 4 mg per ml (24 h); prostate enzyme, 0.05 mg per ml (25 h); incubation at 30°.

** The values are expressed in moles of isomer per 100 moles of total nucleotide synthesized.

*** The 5'-isomers were split by snake venom 5'-nucleotidase and the 3'-isomers by barley *b*-nucleotidase. This last enzyme hydrolyzed 3'-thymidylic acid only very slowly. The isomers were separated by ion-exchange chromatography on Dowex-2 columns, 10 cm × 1.1 cm^2, 150–300 mesh, after removal of phenol from the digests by extraction with ether. The eluent solutions were: for cytidylic acids, 0.05 *M* acetic acid – 0.05 *M* sodium acetate[24]; for adenylic acids, 0.1 *M* formic acid[25]; for thymidylic acids, 0.005 *M* formic acid – 0.05 *M* sodium formate. Flow rate, 0.3 ml per min.

§ The isomers were separated only by paper chromatography, where the 3'- and 2'-nucleotides cannot be distinguished with the solvent used.

TABLE VII

DONOR SPECIFICITY OF THE PHOSPHOTRANSFERASES*

Donor	*Malt enzyme*			*Liver enzyme*			*Prostate enzyme*		
	Incubation	*P***	*P.R.*	*Incubation*	*P***	*P.R.*	*Incubation*	*P***	*P.R.*
	h			h			h		
Phenylphosphate	3	14.0	0.031	4.5	9.9	0.151	0.6	12.8	0.045
5'-Adenylic acid	18	11.0	0.166	18	12.7	0.054	3	13.1	0.013
3'-Adenylic acid***	3	14.8	0.009	18	15.5	0.034	0.75	14.1	0.041
Ribose-5-phosphate	20	12.3	0.026	20	4.8	0.050	1.5	10.4	0.017
Glucose-1-phosphate	30	7.6	0.004	20	6.6	0.035	4	9.6	0.010
β-Glycerophosphate	18	12.8	0.011	18	14.3	0.047	1.5	13.8	0.014

* Experimental conditions: Donor, 38 μmoles per ml; cytidine, 40 μmoles per ml; malt enzyme, 2 mg per ml; liver enzyme, 3 mg per ml; prostate enzyme, 0.05 mg per ml; acetate buffer 0.1 *M*, pH 5.2; 30°.

** Micromoles per ml of inorganic phosphate formed.

*** Mixture of the 3'- and 2'-isomers.

* In view of recent evidence[22,23], we have represented the *a* and *b* nucleotides as the 2'- and 3'-isomers respectively.

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Donors. Table VII shows the effect of several phosphate donors on the P.R. values obtained with the three enzymes. The remarkable specificity of the 5'-nucleotides as donors in the malt system is detailed in Table VIII. The pyrophosphate group of adenosine triphosphate does not seem to have any effect, since the phosphorylation observed can be entirely accounted for by the 5'-adenylic acid liberated from ATP.

It might be mentioned that, with the prostate enzyme, the 5'- and 3'-isomers were produced only with phenylphosphate and 3'-adenylic acid as donors. The other donors seemed to yield only the 5'-isomer.

TABLE VIII

NUCLEOTIDES AS DONORS FOR THE MALT ENZYME*

Acceptor	*Donor*	*μmoles/ml*	*Inorganic P μmoles/ml*	*Nucleotide formed μmoles/ml*	*P.R.*
Cytidine	5'-Inosinic acid	38	12.2	2.41	0.20
	5'-Adenylic acid	36	12.1	2.45**	0.20
	Deoxyadenylic acid	21	12.4	1.67**	0.13
	ATP	31	72.9***	2.95**	0.27***
Uridine	5'-Cytidylic acid	34	12.1	4.57	0.38
	Deoxycytidylic acid	27	11.3	4.65	0.41
	3'-Cytidylic acid§	37	13.9	0.04	0

* Experimental conditions: 40 μmoles/ml of acceptor; 0.1 *M* acetate buffer, pH 5.2; 2 mg per ml of enzyme (1 mg per ml in the case of 3'-cytidylic acid); incubation time, 17 h, 30°.

** Appreciable amounts of inosinic acid were also formed, but, since it could have arisen by direct deamination of adenylic acid, it is not included.

*** Since at the completion of this experiment no pyrophosphate P was left, 62 μmoles of inorganic P must have been contributed by ATP and 10.9 μmoles by 5'-adenylic acid. The calculation of P.R. is based on the latter figure.

§ Mixture of the 3'- and 2'-isomers.

DISCUSSION

The results presented here demonstrate that various tissues contain nucleoside phosphotransferases, enzymes capable of catalyzing the synthesis of mononucleotides. These agents can be provisionally grouped into three classes, according to their specificity characteristics as regards the phosphate donors, the acceptor nucleosides, and the type of nucleotides produced. They are exemplified here by (a) the enzyme first found in malt, but occurring also in wheat; (b) the enzyme prepared from rat liver; (c) the enzyme occurring, in a very active form, in human prostate. Preliminary findings on the distribution of the nucleoside phosphotransferases, to be published soon, tend to indicate that the enzymes are present in all tissues. Plants and bacteria apparently contain the type of enzyme present in malt, and mammals the enzyme present in rat liver.

It would be of great interest to know the extent to which these nucleoside transferases are involved in the formation of cellular nucleotides and nucleic acids. The wide occurrence of these enzymes and the large variety of nucleotides that they are able to produce point to an important role. But a real decision will hardly be possible before the localization of the various agents entering into the phosphate economy of the cell is better known. Though the presence in tissue extracts of hydrolytic enzymes tends to obscure the synthetic capacities and makes necessary the employment of high

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acceptor concentrations, it is quite possible that the spatial separation within the cell of hydrolysis and transfer would obviate these requirements. Even if both activities are carried by the same enzyme, a high local concentration of nucleosides or other mechanisms inhibiting hydrolysis could operate in favor of transfer.

A decision would be facilitated if it were known whether phosphotransferases should be considered as phosphatases or whether at least some of the first mentioned enzymes were solely concerned with the transport of the phosphoryl group from a hydroxyl of one organic substance to that of another. The nucleoside phosphotransferases have their pH optimum around 5.2 and may best be compared with the phosphomonoesterases of type II[26]. The following possibilities could be considered: (a) Transfer and hydrolysis are performed by the same enzyme, with water as the acceptor in the absence of, or in competition with, a nucleoside. (b) The same protein transfers in the presence, and hydrolyzes in the absence, of a coenzyme. (c) The two activities are carried by different enzymes. Though the similarity of the pH-activity curves (Fig. 3) and the inhibition of both reactions by inorganic phosphate to the same extent would seem to favor (a), preliminary fractionation experiments with enzyme preparations from wheat, which have resulted in a partial separation of the two activities, speak against it. A decision between (b) and (c) will have to await the availability of highly purified preparations of nucleoside phosphotransferases.

The efficiency of the transfer reaction depends on the nature of the donor. From our data, it appears, as GREEN AND MEYERHOF already had concluded with regard to the phosphorylation of alcohols and sugars[8], that the phosphate bond energy is not the determining factor. The specificity of phosphate transfer is best brought out when the nucleotides acting as donors for the malt enzyme are considered. Both the position of the phosphate on the sugar moiety and the presence of a purine or pyrimidine in glycosidic linkage appear to be important (see Tables VII and VIII). This property of the malt enzyme suggests that it may function as an agent catalyzing the interconversion of nucleotides and thus play a role similar to that of the transaminases in amino acid metabolism. As for the liver and prostate enzymes, the most efficient donor found so far is a substance that probably does not occur in the organism, namely phenylphosphate. This leaves the nature of the actual donors still in the dark. It should be of interest to test the behavior of phosphoproteins.

The discovery of the nucleoside transferases suggests a pathway of biosynthesis of nucleic acids in which the nucleosides are intermediates. This is not entirely unreasonable since enzymes producing nucleosides from the free bases are widely distributed[27,28]. Results with labeled pyrimidine nucleosides as precursors in the rat[29] are compatible with this pathway; and the fact that cytidine is a much better precursor than uridine could be brought in line with the relative extents of phosphorylation of these two compounds here observed with the liver enzyme. The data on purine derivatives[29] seem to favor a mechanism involving the direct condensation of the base and a ribose-5-phosphate derivative, for which enzymes have recently been found[5,6]. It is quite likely that several pathways are available for the formation of each type of nucleotide.

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SUMMARY

Various tissues contain enzymes catalyzing the transfer of phosphoric acid from low-energy organic phosphates to nucleosides, thereby effecting the synthesis of nucleotides.

The behavior of the enzyme present in malt is studied with respect to pH, acceptor and donor concentrations, and inorganic phosphate effects.

The enzymes, for which the designation nucleoside phosphotransferases is proposed, appear to be specific for nucleosides.

Three types of enzymes are described that differ in their specificities with respect to donors, acceptors and nucleotide isomers formed.

The possible role of the nucleoside phosphotransferases in the biosynthesis of nucleic acids is discussed.

RÉSUMÉ

Divers tissus renferment des enzymes qui catalysent le transfert de l'acide phosphorique entre des phosphates organiques pauvres en énergie et des nucléosides, réalisant ainsi la synthèse de nucléotides.

L'influence du pH, des concentrations en accepteurs et en donateurs et du phosphate minéral sur l'enzyme présent dans le malt a été étudiée.

Ces enzymes, que les auteurs proposent d'appeler nucléoside phosphotransférases, sont spécifiques pour les nucléosides.

Trois types d'enzymes sont décrits, qui diffèrent par leurs spécificités vis à vis des donateurs, des accepteurs et des nucléotides isomères formés.

Le rôle possible de ces nucléoside phosphotransférases dans la biosynthèse des acides nucléiques est discuté.

ZUSAMMENFASSUNG

Verschiedene Gewebe enthalten Enzyme, die die Übertragung der Phosphorsäure aus energetisch niedrigen organischen Phosphatverbindungen auf Nukleoside katalysieren und dabei die Synthese von Nukleotiden bewirken.

Das Verhalten des Enzyms, das im Malz anwesend ist, wird studiert im Hinblick auf den pH, die Akzeptor- und Donor-Konzentration und anorganische Phosphatwirkung.

Die Enzyme, für die die Bezeichnung Nukleosidphosphotransferasen vorgeschlagen wird, scheinen für Nukleoside spezifisch zu sein.

Drei Arten von Enzymen werden beschrieben, die sich in ihrer Spezifität gegenüber Donor und Akzeptor wie auch in den von ihnen gebildeten Nukleotidisomeren unterscheiden.

Die Möglichkeit, dass die Nukleosidphosphotransferasen eine Rolle bei der Biosynthese der Nukleinsäuren spielen, wird diskutiert.

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