THE EFFECT OF ACCEPTOR MODIFICATION UPON THE SYNTHESIS OF DINUCLEOSIDE PHOSPHATES CATALYZED BY NON-SPECIFIC RIBONUCLEASES OF *Penicillium claviforme**

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Received April 6th, 1978

The present paper studies the effect of the modification of heterocyclic base, sugar moiety and the presence of phosphate group on the nucleoside acceptors in the synthesis of dinucleoside phosphates from adenosine 2′,3′-cyclic phosphate as donor, catalyzed by nonspecific acidic extracellular and intracellular ribonucleases from *Penicillium claviforme*. The enzyme binds specifically the acceptor molecule, preferring cytosine nucleosides. It requires the presence of the whole sugar moiety, an exact mutual orientation of the heterocyclic base and the reaction center (5′-hydroxy group), and a suitable conformation of the acceptor molecule. The enzyme–acceptor bond is homochiral and the presence of the N^3—H group in the pyrimidine ring is important. The reaction between the donor and the acceptor is bimolecular and is competitively inhibited by some purine nucleosides.

From the point of view of classification and analytical applicability, the principal property of "ribonucleases" (*i.e.* cyclizing 2′-ribonucleotidyl transferases) is regarded to be their ability of specific splitting of the internucleotidic bonds in ribonucleic acids and oligoribonucleotides under the formation of mono- or oligonucleotides with the terminal 3′-phosphate. Equally important, however, is also their ability to catalyse the reverse reaction, *i.e.* the stereospecific reaction of ribonucleoside 2′,3′-cyclic phosphates with an alcoholic component, *e.g.* aliphatic alcohols or nucleosides, leading to alkyl esters of ribonucleoside 3′-phosphates with the former, or forming stereospecifically products with the (3′→5′)-internucleotidic bond with the latter compounds. Contrary to the cleavage of internucleotidic bonds which has been kinetically treated many times, this synthetic reaction type is complicated and can be expressed by the following simplified form:

$$\begin{array}{l} X > p \\ \downarrow \\ Xp \end{array} + Y \rightleftarrows XpY ,$$

where X > p is a 2′,3′-cyclic phosphate, Xp is a 3′-nucleotide, Y is an alcoholic component and XpY is the reaction product. Such reaction type has not been hitherto treated kinetically.

* Part CXCVII in the series Nucleic Acid Components and Their Analogues; Part CXCVI: This Journal *44*, 593 (1979).

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The general conditions, characterizing the synthetic reaction, are low temperature, high concentration of the reactants, small enzyme concentration and an excess of the alcoholic component (acceptor) relative to the 2',3'-cyclic phosphate (donor). In addition to these factors, the reaction is controlled by stereospecificity of the enzyme with respect to the donor and by the ratio of rate constants; synthetically the most advantageous are enzymes with a suppressed ability to hydrolyse the cyclic phosphate which afford high yields of the products XpY (ref.[1-3]).

The microorganism *Penicillium claviforme* releases into the medium several RNases: (ref.[4]) the nonspecific RNase of *P. claviforme* is an extracellular enzyme of molecular weight 37 000 and acidic pH-optimum; it is similar *e.g.* to the RNase T2 from *Aspergillus oryzae*[5], to the *P. brevicompactum* RNase[6] *etc.* As shown by preliminary experiments, this RNase can also catalyse the synthesis of internucleotidic bonds. The intracellular nonspecific RNase of *P. claviforme* (mol. weight 36000) exhibits also high hydrolase activity and liberates nucleotides from RNA in the order A > G > U > C (see[7]). Also this enzyme can catalyze the synthetic reaction between a 2',3'-cyclic phosphate and nucleoside acceptors.

Preliminary experiments on a limited number of acceptors have surprisingly shown that both these enzymes are not only specific towards the donor but also towards the acceptor of the nucleotide moiety[8]. The present work studies in detail the effect of modifications of the acceptor molecule upon the course of the dinucleoside monophosphate synthesis. Adenosine 2',3'-cyclic phosphate, as the best of "natural" donors, was used and both intra- and extracellular RNases were studied simultaneously. Because of the difficult kinetic analysis only three readings were made during each run, the donor : acceptor ratio being kept 1 : 3. It was assumed that after 24 h an equilibrium was established.

EXPERIMENTAL

Enzymes Employed

The extracellular acidic nonspecific RNase from *P. claviforme* (m.w. 37 000, pH_{opt} 6·5 for RNA, 4·5—5·0 for uridine 2',3'-cyclic phosphate) was isolated according to ref.[4]. The intracellular acidic nonspecific RNase from *P. claviforme* (PCI) (m.w. 36 000—38 500, p*I* = 5·0, pH_{opt} 6·0) for RNA, 4·0 for uridine 2',3'-cyclic phosphate, 4·5—5·0 for adenosine 2',3'-cyclic phosphate) was isolated according to ref.[7]. Determination of RNase activity (ref.[9]): 0·05M Sodium acetate solution (pH 5·0; 0·4 ml) and the appropriately diluted enzyme solution (0·4 ml) were added to a solution of the yeast RNA in the same buffer (0·4 ml, 8 mg/ml). After incubation at 25°C for 25 min, 0·75% uranyl acetate solution (0·5 ml) in 25% perchloric acid was added and the mixture was made up to 2 ml with water. After standing at 0°C for 30 min the mixture was filtered, 0·1 ml aliquote of the filtrate was made up to 3 ml with water and the extinction at 260 nm was measured. In a control experiment, the enzyme solution was added after the precipitation with uranyl acetate. One enzyme unit corresponded to the optical density 1 at 260 nm.

Synthesis of Dinucleoside Monophosphates and Dinucleotides

The incubation mixture contained 12·5 μmol of adenosine 2',3'-cyclic phosphate (lithium salt, chromatographically and electrophoretically homogeneous) and 37·5 μmol of the nucleoside (acceptor) in 0·05M sodium acetate–acetic acid buffer (0·1 ml; pH 5·0), containing 1·4 units of RNase. The mixture was incubated at 0°C, 5 μl aliquots were taken after 1, 4 and 24 h and analysed by paper electrophoresis on a Whatman No 3 MM paper in 0·1M triethylammonium hydrogen carbonate (pH 7·5) at 20 V/cm (or, in some cases, by paper chromatography in 2-propanol–ammonia–water 7 : 1 : 2 on the same paper). The spots of the starting compound, product, and adenosine 3'-phosphate were eluted with 0·1M-HCl and absorbance at 260 nm was measured (Table I, II). In the calculation, the molar extinction coefficient for adenosine was taken as 14000, the extinction coefficients of the nucleosides were calculated for 260 nm, the hyperchromicity being disregarded (Table III).

RESULTS AND DISCUSSION

Three aspects of the chemical modification of nucleoside acceptors in the transfer reaction, catalysed by both the non-specific ribonucleases from *P. claviforme*, were studied: effect of the heterocyclic base structure in the nucleoside, effect of variation in the sugar part of the acceptor molecule and, finally, effect of presence of the charged phosphomonoester group in the acceptor molecule. All these three aspects can influence significantly the possible practical use of the enzymes for the preparative purposes.

Effect of Heterocyclic Base Modification

From the data in Table I the following conclusions can be drawn: *a*) Pyrimidine nucleosides are generally better substrates than purine nucleosides. A detailed study in the series of purine nucleosides would be difficult since these compounds are only sparingly soluble. For this reason *e.g.* guanosine derivatives could not be tested and some adenosine derivatives were measured only in saturated solutions. *b*) In the pyrimidine nucleosides series the enzyme exhibits an extraordinary activity towards the cytidine derivatives as compared with derivatives of uridine and similar compounds. Since the 2-pyridone, 2-pyrimidone and 4-amino-6-pyrimidinone derivatives *VII*, *VIII* and *X* show practically no acceptor activity, we can assume that the structural unit, necessary for the existence of acceptor activity, can be represented by the formula A which corresponds both to uracil and cytosine nucleosides. This is also in accord with the fact that the reaction fails with isocytidine (*XVI*). However, since the 3-isomer of uridine *IX* is a substantially better substrate than uridine itself (*I*) whereas the already mentioned 2-pyrimidinone derivative *VIII* is unreactive, it cannot be excluded that the position of the N^3—H group in the uracil ring plays the decisive role. This assumption is confirmed also by a decrease in acceptor ability of uridine caused by methylation in the position N^3 (*IV*) and first of all by the marked decrease in activity of cytidine due to the successive methylation of the 4-amino group:

TABLE I

Enzymatically Catalyzed Synthesis of Dinucleoside Phosphates from Adenosine 2',3'-Cyclic Phosphates and Base-Modified Ribonucleoside

	Nucleoside (N)	t h	RNase *P. claviforme* extracelular ApN, %	extracelular Ap, %	intracelular ApN, %	intracelular Ap, %
None		1	—	61·0	—	74·6
		4	—	87·4	—	98·8
		24	—	100	—	100
I	Uridine	1	3·6	24·7	4·1	25·8
		4	5·4	42·2	5·7	44·0
		24	7·5	80·0	7·3	74·3
II	5-Methyluridine	1	2·6	51·2	3·8	47·4
		4	4·2	73·8	5·1	74·0
		24	—	—	14·7	75·0
III	6-Methyluridine	1	2·6	63·8	1·6	74·7
		4	2·7	86·3	2·4	90·1
IV	3-Methyluridine	1	—	—	2·4	57·1
		4	—	—	2·8	83·0
		24	—	—	2·0	98·0
V	5-Bromouridine	1—24	—	—	+[a]	100
VI	5-Aminouridine	1—24	—	—	+[a]	100
VII	1-*Rf*-2-Pyridone[b]	1	—	—	0·5	62·0
		4	—	—	0	89·8
VIII	1-*Rf*-2-Pyrimidinone[b]	1	—	—	1·9	54·6
		4	—	—	3·6	77·5
IX	3-*Rf*-Uracil	1	15·0	54·6	13·8	57·2
		4	12·6	73·8	12·0	74·0
		24	4·0	95·0	0	100
X	1-*Rf*-4-Amino-6-pyrimidinone	1	—	—	0	69·8
		4	—	—	0	89·8
XI	6-Azauridine	1—24	+[a]		+[a]	
XII	Cytidine	1	23·6	12·9	23·2	14·4
		4	33·4	22·6	35·2	24·4
		24	33·3	45·3	29·2	51·0
XIII	5-Bromocytidine	1	11·6	36·1	8·1	37·2
		4	12·7	57·3	14·3	57·3
		24	12 9	77·6	—	—
XIV	4-Methylcytidine	1	13·0	46·9	12·4	52·1
		4	16·5	66·2	13·5	72·2
		24	8·0	89·4	10·2	85·0

TABLE I
(*Continued*)

	Nucleoside (N)	*t* h	RNase *P. claviforme* extracelul. ApN, %	extracelul. Ap, %	intracelul. ApN, %	intracelul. Ap, %
XV	4,4-Dimethylcytidine	1	1·4	41·0	3·0	38·9
		4	2·5	62·5	4·5	63·4
		24	4·0	92·1	7·2	83·3
XVI	Isocytidine	1	—	47·5	3·8	46·6
		4	1·7	77·0	3·5	79·0
XVII	6-Azacytidine	1	—	—	12·0	54·4
		4	—	—	10·6	77·0
XVIII	9-*Rf*-Purine[b]	1	18·3	33·9	9·4	48·0
XIX	Adenosine	1	4·3	63·2	3·3	63·7
		4	3·1	85·6	2·2	88·9
		24	0	100	0	100
XX	1-Methyladenosine	1	13·7	59·3	18·3	54·8
		4	10·2	80·0	8·5	81·5
		24	+[a]	100	1·2	96·4
XXI	6-Methyladenosine	1	2·9	4·5	1·9	4·0
XXII	2-Aminoadenosine	1	0	79·6	0	81·2
XXIII	Adenosine N_3-oxide	1	2·0	64·1	3·3	61·5
XXIV	8-Chloroadenosine	1	0	66·0	0	60·0
XXV	8-Aminoadenosine	1	0	45·9	0	47·4
XXVI	8-Methylaminoadenosine	1	0	70·0	0	65·5
XXVII	8-Dimethylaminoadenosine	1	0	66·2	—	—
XXVIII	8-Hydroxyadenosine	1	9·6	59·2	6·1	61·5
XXIX	8-Methoxyadenosine	1	0	23·4	—	—
XXX	8-Thioadenosine	1	2·4	51·8	1·8	56·0
XXXI	8-Methylthioadenosine	1	+[a]	66·5	0	61·7
XXXII	Adenosine 8-sulfonic acid	1	+[a]	—	—	—
XXXIII	Isoadenosine	1	0	40·2	—	—
XXXIV	9-*Rf*-6-Hydroxylaminopurine[b]	1	3·0	68·1	3·2	63·8
XXXV	Inosine	1	3·8	63·8	0·6	67·6
		4	3·7	85·1	0·5	95·0
XXXVI	1-Methylinosine	1	9·4	57·0	7·8	56·0
		4	9·5	78·8	8·8	78·9

Traces; [b] *Rf* β-D-ribofuranosyl residue.

TABLE II

Effect of Sugar Modification of the Nucleoside Acceptor on the Enzymatically Catalysed Dinucleoside Monophosphate Synthesis from Adenosine 2',3'-Cyclic Phosphate

	Nucleoside	*t* h	RNase *P. claviforme*			
			extracellular		intracellular	
			ApN, %	Ap, %	ApN, %	Ap, %
XXXVII	2'-Deoxyuridine	1	2·8	42·2	4·0	33·8
		4	5·3	61·6	3·6	65·0
		24	4·5	92·1	—	—
XXXVIII	2'-Deoxycytidine	1	20·8	42·9	15·0	31·5
		4	20·2	62·8	18·4	65·0
		24	8·2	87·0	—	—
XXXIX	5-Methyl-2'-deoxycytidine	1	—	—	18·9	28·7
		4	—	—	24·5	54·0
XL	3'-Deoxycytidine	1	9·4	2·9	11·0	1·5
		4	20·6	5·0	21·0	16·9
		24	38·5	20·5	40·6	20·0
XLI	1-(β-D-Xylofuranosyl)cytosine	1	8·5	30·5	9·1	32·5
		4	8·7	50·2	8·4	56·2
XLII	1-(β-D-Arabinofuranosyl)cytosine	1	9·1	50·0	18·1	55·0
		4	12·3	76·0	9·4	81·1
		24	0	100	—	—
XLIII	2',3'-O-Isopropylidenecytidine	1	20·9	43·8	21·5	45·0
		4	19·5	62·5	16·0	68·0
		24	3·9	92·2	3·5	93·0
XLIV	1-(2-Deoxy-α-D-ribofuranosyl)-cytosine	1	1·9	30·5	0	41·0
		4	2·6	41·9	0	52·0
		24	+[a]	—	4·5	81·8
XLV	L-Cytidine	1	2·7	44·2	5·4	47·2
		4	4·4	67·7	6·2	73·0
		24	0	99·1	—	—
XLVI	1-(*R,S*)-(2,3-Dihydroxypropyl)-cytosine	1	0	27·9	0	30·4
		4	0	46·6	0	49·2
		24	0	83·9	0	100
XLVII	2'-O-Methyladenosine	1	6·5	69·1	6·7	67·0
XLVIII	9-(β-D-Xylofuranosyl)adenine	1	16·5	37·1	19·2	35·1
XLIX	9-(α-L-Lyxofuranosyl)adenine	1	1·4	63·5	1·6	64·6
L	L-Adenosine	1	1·5	67·0	2·5	64·0

TABLE II
(*Continued*)

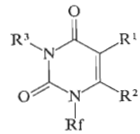
	Nucleoside	*t* h	RNase *P. claviforme* extracellular ApN, %	extracellular Ap, %	intracellular ApN, %	intracellular Ap, %
LI	9-(β-D-Ribopyranosyl)adenine	1	0	55·0	0	57·0
LII	Uridine 3′-phosphate	1	3·6	24·7	4·1	25·8
		4	5·4	42·2	5·7	44·0
		24	11·9	74·9	12·9	78·2
LIII	Cytidine 3′-phosphate	1	16·9	18·4	13·5	10·2
		4	25·0	23·1	27·6	14·9
		24	27·7	44·6	35·1	37·9

[a] Traces.

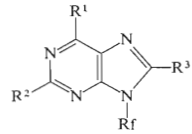
the monomethyl derivative *XIV* is capable of formation of the imino form B, containing the N^3—H bond, in the dimethyl derivative *XV* such a form is not possible and the compound is inactive. Also in the 2′-deoxyribonucleoside series, the cytosine derivatives *XXXVIII* and *XXXIX* (Table II) are better acceptors than the uracil derivative *XXXVII*.

Also isoadenosine (*XXXIII*) can be, in a way, regarded as a pyrimidine derivative; however, this compound has no N^3—H group available for the interaction with the enzyme and its acceptor activity fails.

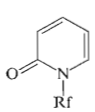
Introduction of a methyl group into the position 5 or 6 (compounds *II*, *III*, *XXXIX*) or an amino group into the position 5 (*VI*) of the pyrimidine ring does not apparently influence the acceptor activity whereas the presence of a 5-bromo substituent (*V*, *XIII*) causes an activity decrease, perhaps as a result of greater acidity of the heterocyclic nucleus. Also the lower activity of both the 6-aza derivatives *XI* and *XVII* which are more acidic than the parent uracil and cytosine derivatives supports this assumption. *c*) Although the choice of the purine nucleosides is limited by their solubility and the interpretation is complicated by low overall reaction yields, we can conclude that the only good acceptor in this series appears to be the unsubstituted purine ribonucleoside (*XVIII*). The presence of amino (*XIX*), methylamino (*XXI*) hydroxy (*XXXV*) or hydroxylamino (*XXXIV*) group in the position 6 causes a decrease in acceptor activity. A further decrease is caused by substitution with an amino group in the position 2 (*XXII*), or various substituents in the position 8 (*XXIV* to *XXVII*, *XXIX*–*XXXII*). In contrast, 8-hydroxyadenosine (*XXVIII*) is a better



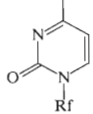
I, $R^1 = R^2 = R^3 = H$
II, $R^1 = CH_3$, $R^2 = R^3 = H$
III, $R^2 = CH_3$, $R^1 = R^3 = H$
IV, $R^3 = CH_3$, $R^1 = R^2 = H$
V, $R^1 = Br$, $R^2 = R^3 = H$
VI, $R^1 = NH_2$, $R^2 = R^3 = H$



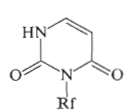
XVIII, $R^1 = R^2 = R^3 = H$
XIX, $R^1 = NH_2$, $R^2 = R^3 = H$
XXI, $R^1 = NHCH_3$, $R^2 = R^3 = H$
XXII, $R^1 = R^2 = NH_2$, $R^3 = H$
XXIV, $R^1 = NH_2$, $R^2 = H$, $R^3 = Cl$
XXV, $R^1 = R^3 = NH_2$, $R^2 = H$
XXVI, $R^1 = NH_2$, $R^2 = H$, $R^3 = NHCH_3$
XXVIII, $R^1 = NH_2$, $R^2 = H$, $R^3 = OH$
XXIX, $R^1 = NH_2$, $R^2 = H$, $R^3 = OCH_3$
XXX, $R^1 = NH_2$, $R^2 = H$, $R^3 = SH$
XXXI, $R^1 = NH_2$, $R^2 = H$, $R^3 = SCH_3$
XXXII, $R^1 = NH_2$, $R^2 = H$, $R^3 = SO_3H$
XXXIV, $R^1 = NHOH$, $R^2 = R^3 = H$
XXXV, $R^1 = OH$, $R^2 = R^3 = H$



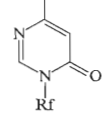
VII



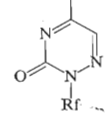
VIII, R = H
XII, R = NH_2
XIV, R = $NHCH_3$
XV, R = $N(CH_3)_2$



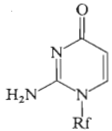
IX



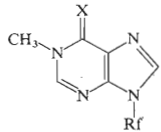
X



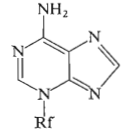
XI, R = OH
XVII, R = NH_2



XVI

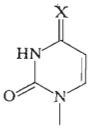


XX, X = NH
XXXVI, X = O

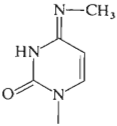


XXXIII

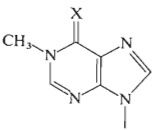
In formulae *I* – *XXXV*, Rf = β-D-ribofuranosyl residue



A



B



C

TABLE III

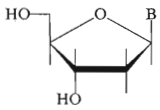
UV-Spectra of Dinucleoside Monophosphates ApN and Dinucleotides ApNp (pH 2·0)

Acceptor N No	λ_{max}, nm	λ_{min}, nm	$A\frac{250}{260}$	$A\frac{270}{260}$	$A\frac{280}{260}$	$A\frac{290}{260}$
I	258	232	0·88	0·67	0·54	0·21
II	260	232	0·63	0·77	0·43	0·17
III	256	230	0·93	0·59	0·24	0·07
IX	255	236	1·00	0·73	0·46	0·24
XII	276	236	0·73	1·00	0·80	0·50
XIII	256, 300	240	0·93	0·84	0·61	0·55
XIV	263	238	0·78	0·95	0·73	0·49
XV	282	242	0·70	1·37	1·64	1·59
XIX	256	233	0·90	0·80	0·04	0·01
XX	257	233	0·90	0·68	0·32	0·16
XXIII	256	232	1·07	0·77	0·39	0·23
XXVIII	260	233	0·92	0·95	0·39	0·08
XXXV	250	224	1·30	0·47	0·22	0·11
XXXVI	255	232	1·08	0·69	0·34	0·17
XXXVII	258	232	0·89	0·61	0·22	0·09
XXXVIII	263	235	0·76	0·98	0·69	0·38
XXXIX	263, 286	236	0·80	0·92	0·75	0·64
XL	263	235	0·78	0·93	0·71	0·40
XLI	262	233	0·83	0·95	0·67	0·38
XLII	265	235	0·72	0·97	0·74	0·43
XLIII	263	248	0·75	0·95	0·74	0·46
XLVII	257	233	1·00	0·67	0·45	0·28
XLVIII	260	235	0·77	0·77	0·32	0·11
LII	257	234	0·89	0·78	0·39	0·19
LIII	263	235	0·79	0·97	0·72	0·46

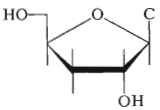
substrate than adenosine (*XIX*), perhaps as the result of the possible formation of a —$C^8(=O)$—N^7H— grouping, similar to the uracil system. The increase in the acceptor ability in both adenosine and inosine by methylation at N_1 in compounds *XX* and *XXXVI* is very interesting: this substitution brings about a fixation of the structure of the type C, containing the respective oxo and imino forms in the position 6, and also enhances the basicity of the nucleus.

Effect of Sugar Part of the Acceptor

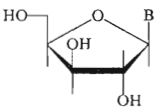
The acceptor ability of a nucleoside depends not only on the presence of a sugar moiety but also on its configuration: since 1-(*RS*)-(2,3-dihydroxypropyl)cytosine (*XLVI*), which contains a primary hydroxyl and can exist in a nucleoside-like conformation[10], shows no acceptor properties, it is obvious that the presence of an intact sugar moiety is substantial. Since, further, L-cytidine (*XLV*), as well as L-adenosine (*L*), also lack a marked acceptor activity compared with the corresponding D-enantiomers *I* and *XII*, it is also obvious that the enzyme–acceptor interaction is of homochiral type. The importance of the strict mutual orientation of the reaction center (5′-hydroxy group) and the binding center (base), following from the above conclusion is further supported by the following observations: the α-anomer of 2′-deoxycytidine (*XLIV*) possesses almost zero acceptor activity because the mutual orientation of these centers does not correspond to the requirements of the enzyme; also the α-L-lyxofuranosyl derivative *XLIX*, differing from the ribonucleoside in the configuration of the 4′-hydroxymethyl group (reaction center), is less active than the compound *XIX*, finally, the β-D-ribopyranoside *LI*, isomeric with the compound *XIX*, exhibits no acceptor properties – it does not contain a primary hydroxy group at the (reaction) center. The absence of a hydroxyl at the position 2′ or 3′ does not reduce seriously the acceptor activity: both the 2′-deoxyribonucleosides *XXXVII* and *XXXVIII* are as good substrates as the ribonucleosides *I* and *XII*, and the 3′-deoxyribonucleoside *XL* is as good acceptor as the 2′-deoxyribonucleoside *XXXVIII* (because of different hydrolysis rates of the products, arising from both compounds, the pseudo-equilibrium states for both compounds are different). The acceptor ability is not affected by blocking of one (compound *XLVII*) or both hydroxy groups (compound *XLIII*) in the positions 2′ and 3′ of the ribonucleoside. An important factor, however, appears to be the configuration of these hydroxy groups which alters the stereochemistry (and the conformation) of the nucleosides: both the β-D-arabino- and β-D-xylofuranosyl derivatives *XLII* and *XLI*, respectively, are worse substrates than cytidine (*XII*). Moreover, in the xylofuranosyl derivatives the mutual interaction between the reaction center and the neighbouring vicinal *cis*-3′-hydroxy group ought to enhance the reactivity of the 5′-hydroxy group (see compound *XLVIII*) *via* the intramolecular hydrogen bond formation.



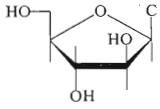
XXXVII, B = uracil
XXXVIII, B = cytosine
XXXIX, B = 5-methylcytosine



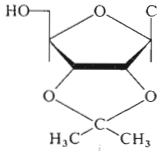
XL



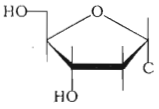
XLI, B = cytosine
XLVIII, B = adenine



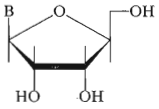
XLII



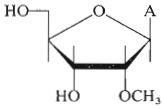
XLIII



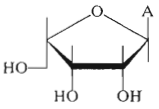
XLIV



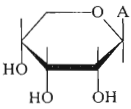
XLV, B = cytosine
L, B = adenine



XLVII



XLIX



LI

In formulae *XLII*–*XLIV*, *XLVII*, *XLIX* and *LI*, C cytosine, A adenine residue.

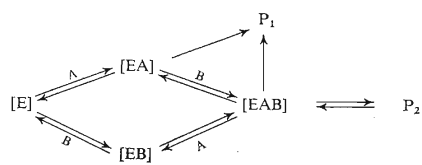
Effect of the Phosphomonoester Group

This effect was followed using uridine 3′-phosphate (*LII*) and cytidine 3′-phosphate (*LIII*): during the initial stage both compounds exhibit the same acceptor activity

as the parent ribonucleosides and during the reaction the yield of products slightly increases. We assume that this apparent increase is not due to an enhanced activity but to slower reverse hydrolysis of the reaction product; esterification of the 3′-hydroxy group does not seem to exhibit any substantial effect on the nucleoside acceptor ability.

In all the above-mentioned observations one has to consider the effect of the reverse hydrolytic reaction which reduces the amount of product and increases the amount of adenosine 3′-phosphate. It is obvious from Tables I and II that the synthetic reaction simultaneously inhibits the hydrolysis A > p → Ap. This reverse hydrolysis can cause an incorrect interpretation in such cases when its rate is significantly higher than usual. Since we can properly distinguish only such case in which the reverse reaction is markedly slower (see compound *XL*) than that of the standard (cytidine), we cannot exclude that in extreme cases the interpretation is not completely correct. By comparison with the control experiments in the presence or absence of cytidine, additional effects of modified acceptors can be followed: thus, *e.g.*, the data in Table I indicate a high inhibitory activity of some compounds, having the character of a simultaneous inhibition of hydrolysis and synthesis (*e.g.* compounds *XXV*, *XXIX*) or of a mere inhibition of hydrolysis (*e.g.* compound *XXI*).

The most important conclusion, drawn from the above results, consists in the specificity of both the acidic ribonucleases of *P. claviforme* towards the absolute configuration, stereochemistry, conformation and structure of the heterocyclic base of the acceptor. Both enzymes show low absolute specificity towards natural substrates in the transfer reaction and a somewhat higher specificity in the hydrolytic reaction; however, in their synthetic reaction both enzymes are almost absolutely specific for cytosine nucleosides as acceptors, at least within the natural nucleoside series. This leads to the obvious conclusion that both these ribonucleases are binding the donor (adenosine-2′,3′-cyclophosphate) as well as the acceptor (nucleoside) and that the catalysed reaction is a typical bimolecular reaction involving three types of ES-complexes according to the following scheme where A is donor, B acceptor, P_1 product of the non-reversible hydrolysis, and P_2 product of reversible synthesis. A kinetical treatment of this scheme which would probably allow at least the determination of ratios of some rate or equilibrium constants, would be beyond the scope of this work.



Because only little attention has hitherto been paid to a detailed study of synthetic reactions catalysed with „ribonucleases" we cannot even exclude that also some of the already known enzymes of this group are binding specifically the acceptor molecules. Therefore, in the light of our results we feel that the existing assumption of nonspecific interactions should be reexamined.

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Translated by M. Tichý.