Stereoelectronic effects in RNase-catalysed reactions of dinucleoside phosphate cleavage

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Received 5 September 1984; revised version received 19 October 1984

The rate at which dinucleoside phosphates are cleaved by RNases is supposed to be determined by the mole fraction of enzyme-substrate complexes in which the phosphodiester moiety of a dinucleoside phosphate has a highly reactive conformation. The mole fraction of such complexes for a particular RNase depends on the nature of a nucleoside at the O5′-end of the phosphodiester bond. Experimental data are presented to support this hypothesis.

RNase *Stereoelectronic control specificity*

1. INTRODUCTION

When RNA is hydrolysed by RNase A, the rate of cleavage of the internucleotide bond containing a pyrimidine nucleoside at its O3′-end depends on the nature of a nucleoside at the O5′-end [1]. A pyrimidine–purine bond is cleaved faster than a pyrimidine–pyrimidine bond. This effect was studied in detail in the hydrolysis of dinucleoside phosphates [2,3]. The rate constant for the internucleotide bond cleavage in the series of CpX and UpX (where X is A, G, C or U) was shown to change more than 100-fold whereas the Michaelis constant remained virtually the same. Similar effects produced by the nature of a nucleoside at the O5′-end of the phosphodiester bond were found for the cleavage of dinucleoside phosphates catalyzed by other RNases [4–6]. For these, just as for RNase A, the highest rate of dinucleoside phosphate cleavage is observed for a particular, typical of each enzyme, nucleoside at the O5′-end.

Theoretical analysis of the phosphodiester electronic structure has shown that the energy of the oxygen–phosphorus ester bond depends considerably on the value of torsion angles α and ζ (fig.1) [7,8]. When dinucleoside phosphates are cleaved by cyclizing RNases, the value of angle ζ is determined by the geometry of the phosphoribosyl moiety and the linear arrangement of O2′, P and O5′ atoms which is required in the formation of an intermediate [9]. The value of angle α must be consistent with the minimal energy of the P–O5′ bond at a fixed value of angle ζ. The weak influence of a 5′-nucleoside on the K_m value implies that complexes of each RNase and a dinucleoside phosphate may be heterogenous in their structure. The dinucleoside phosphate is in a highly reactive conformation only in some complexes whose molar

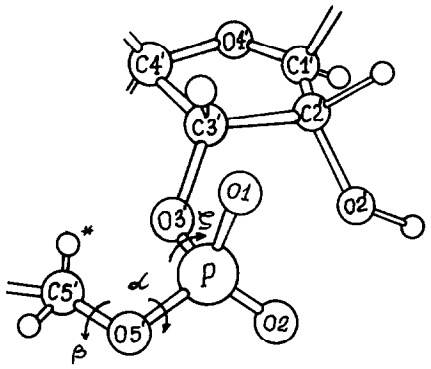

Fig.1. Schematic representation of the phosphodiester moiety of a dinucleoside phosphate in the *cis-gauche*$^+$ conformation. The *pro-S* position of the nucleoside 5′-CH_2 group at the O5′-end of the phosphodiester bond is marked with an asterisk.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

fraction depends on the nature of a nucleoside at the O5′-end of the phosphodiester bond; in other complexes it is less reactive. In that case, the rate at which a particular dinucleoside phosphate is cleaved by RNase depends on the ratio between the mole fractions of two enzyme–substrate complex types.

This paper presents experimental evidence to support the above hypothesis.

2. MATERIALS AND METHODS

Homogeneous pyrimidine-specific RNase A, guanyl-specific *P. brevicompactum* and non-specific *P. brevicompactum* RNases were prepared as in [10–12]. The synthesis of Cyd-P-(*pro-S*-Me)Urd, Cyd-P-(*pro-R*-Me)Urd, Guo-P-(*pro-S*-Me)Urd and Guo-P-(*pro-R*-Me)Urd was described in [13]. These compounds were a generous gift from Dr N.Sh. Padyucova.

The kinetic parameters for the cleavage of modified dinucleoside phosphates were determined using the spectrophotometric and pH-stat techniques. In assaying the concentrations and cleavage rates of dinucleoside phosphates, the molar extinction coefficients of *pro-S* and *pro-R* methyl derivatives and their changes in the reaction of transesterification were taken to equal the corresponding values of natural dinucleoside phosphates given in [14–16]. The kinetic parameters were measured by the spectrophotometric technique in buffer solutions containing 0.05 M Tris–HCl, 0.05 M sodium acetate and 0.1 M sodium chloride. The pH was adjusted by adding acetic acid and measured using a combined GK 2401C electrode (Radiometer, Denmark) and pH-340 pH meter (USSR). Spectral measurements were performed with a Cary-118 spectrophotometer (Varian, USA).

The rates of Cyd-P-(*pro-S*-Me)Urd cleavage were estimated with the aid of a pH-stat. It has been found that the rate constant of transesterification for Cyd-P-(*pro-S*-Me)Urd catalysed by RNase A at pH 6.5 and *P. brevicompactum* RNase at pH 5.7 is far less than the rate constants of Cyd-2′,3′-P hydrolysis. Here, the constants k_{cat} and k_m determined from the rate of Cyd-3′-P formation should be assigned to the transferase step of the reaction. The rate of Cyd-3′-P formation was measured by recording the rate of acid production due to the secondary ionization of the phosphate group. The measurements were done using a Radiometer pH-stat with an automatic ABU-12 burette. The pH of the reaction was maintained constant by adding a 0.56 mM KOH solution to the cuvette. The cuvette was thermostatted at 25°C. The substrate was dissolved in a 0.2 M NaCl solution. The volume of the reaction mixture was 2.0–2.1 ml. The volume of a KOH solution added to maintain the pH constant did not exceed 0.2 ml. In calculating the rate constant, the pK of Cyd-3′-P was taken to be equal to 5.9 [17].

3. RESULTS AND DISCUSSION

As follows from X-ray studies of the RNase A complex with Urd-P-(CH_2)-Ado [18], the phosphodiester moiety of the fast-cleaved substrate Urd-P-Ado in the enzyme–substrate complex of RNase A has mainly a *cis-gauche*$^+$ conformation with an angle $\alpha \approx 0°$. X-ray analysis of Cyd-P-Ado and Urd-P-Urd complexes with an inactive RNase S* derivative prepared by denitrophenylating the ϵ-amino group of Lys^{41} has shown that the 3′-nucleoside and phosphate group of both dinucleoside phosphates are bound at the active site of the enzyme in a similar manner, whereas the binding sites for the 5′-nucleoside are different [19]. Therefore, in the enzyme–substrate complexes of RNase A with such slowly cleaved dinucleoside phosphates as Cyd-P-Urd or Urd-P-Urd, the predominant conformation of their phosphodiester moieties differs from the conformation for a fast-cleaved substrate only due to the value of the torsion angle α. According to the hypothesis formulated in section 1, the rate constant for the cleavage of dinucleoside phosphates catalysed by RNase A (including the slowly cleaved substrates Urd-P-Urd and Cyd-P-Urd) is proportional to the molar fraction of complexes which are structurally similar to the complexes of RNase A with Urd-P-Ado and Cyd-P-Ado.

To check this hypothesis experimentally, we decided to compare the kinetic parameters of enzyme-catalysed reactions for slowly cleaved substrates and their derivatives for which the binding to RNase A in a conformation typical of fast-cleaved substrates is sterically hindered. Such derivatives can be prepared by methylation at the *pro-S* position of the 5′-CH_2 group in a 5′-nucleo-

Table 1

Kinetic parameters for the cleavage of modified dinucleoside phosphates Xp(*pro-S*-Me)Urd and Xp(*pro-R*-Me)Urd catalysed by RNases with different specificity

Enzyme	Substrate	k_{cat} (s^{-1})	$K_m \times 10^4$ (M^{-1})
Pyrimidine-specific	Cp(*pro-S*-Me)Urd	0.032	7.4
RNase A (pH 6.5)	Cp(*pro-R*-Me)Urd	1.9	14.3
Guanyl-specific *P. brevicompactum* RNase (pH 6.5)	Gp(*pro-S*-Me)Urd	0.006	0.33
	Gp(*pro-R*-Me)Urd	1.5	0.33
Non-specific *P. brevicompactum* RNase (pH 5.7)	Cp(*pro-S*-Me)Urd	3.5	2.56
	Cp(*pro-R*-Me)Urd	200	1.85

side. In this case, the conformation of dinucleoside phosphate with $\alpha \approx 0°$ is sterically hindered at the value of angle β ($\beta \approx +150°$) which is recorded for Urd-P-(CH_2)-Ado in the complex [18]. One cannot directly compare the kinetic parameters for the enzyme-catalysed cleavage of the thus modified dinucleoside phosphate and the corresponding unmodified dinucleoside phosphate because their leaving groups (primary and secondary alcohol radicals, respectively) have different pK values. That is why we compared the kinetic parameters with those for the corresponding *pro-R*-methyl derivative.

The conclusion about the *cis-gauche*$^+$ conformation of dinucleoside phosphates being highly reactive in their complexes with RNase A was based on the results of X-ray studies of these enzyme–substrate complexes. However, it seems to hold for other RNases as well. Indeed, for a given ζ value, the value of angle α must be consistent with the minimal energy of the P–O5′ bond to reach the maximal rate at which an intermediate with the penta-coordinate phosphorus atom is formed. The addition of O2′ to the phosphorus when the intermediate is formed involves an elongation of the P–O5′ bond. The intermediate is formed at a highest rate in the case of a conformer whose energy of the P–O5′ bond is minimal. The value found for $\alpha = 0°$ corresponds to the minimal energy of the P–O5′ bond. At $\alpha = 0°$, neither of the orbitals of the O5′ lone electron pairs is antiperiplanar ro the neighbouring P–O bond, which would strengthen the P–O5′ bond [7,8]. In contrast, each of these two orbitals is synperiplanar to the phosphoryl P–O bonds. This should weaken the P–O5′ bond to a considerable extent. That is why we decided to compare the kinetic parameters for the transesterification of *pro-S*-methyl and *pro-R*-methyl derivatives not merely for RNase A, but for other RNases as well.

One can see from table 1 that all of the tested RNases cleave *pro-S*- and *pro-R*-methyl substrate analogs at a quite different rate. Here, the K_m values for each substrate pair differ by less than a factor of 2. However, the catalytic rate constants differ by 60–250 times for the corresponding substrate pair. All the RNases cleave the *pro-S*-methyl derivatives of dinucleoside phosphates at a far lower rate than their *pro-R*-methyl derivatives. These data are consistent with the hypothesis about the stereoelectronic control of dinucleoside phosphate cleavage catalysed by RNases.

ACKNOWLEDGEMENTS

The authors express their thanks to Dr Nelly Sh. Padyukova for her generous gift of *pro-S*-methyl and *pro-R*-methyl derivatives of dinucleoside phosphates, and to Professor Marat Ya. Karpeisky for stimulating discussions.

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