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STRUCTURE AND REACTIVITY OF OLIGONUCLEOTIDES. PART I KINETICS OF THE NON-ENZYMATIC TRANSPHOSPHORYLATION OF ADENYLYL-(3'-5')-ADENOSINE 3'-PHOSPHATE AND OTHER DINUCLEOTIDES

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Kinetics of the non-enzymatic transphosphorylation of several diribonucleotides have been studied. First-order rate constants for the reactions, which are acid-base-catalyzed, have been obtained. Base-sequence and base composition dependent nature of rates is discussed.

For a better understanding of the conformation of ribonucleic acids(RNA) in solution, the conformation and conformational stability of simple oligoribonucleotides of various base composition have been extensively studied.$^{1)}$ The stability of the conformation of a single-stranded polynucleotide in aqueous solution is determined by various forces such as "vertical stacking" between neighboring bases and electrostatic interactions originated from negatively charged phosphate groups.$^{2)}$ Tinoco and his co-workers$^{3,4)}$ have first demonstrated using optical rotatory dispersion(ORD) that dinucleoside monophosphates adopt a dissymmetrical single-stranded helical conformation under certain conditions. The thermal denaturation was also shown to be a non-cooperative process.$^{5)}$ Thus, diribonucleotides of different base composition are suitable models for detailed conformational studies of single-stranded helical polynucleotides by physico-chemical techniques: absorption, ORD, circular dichroic(CD) and nuclear magnetic resonance(NMR) spectral analysis, and X-ray crystallographic method. For this, such studies of dinucleotides have been actively pursued in many laboratories.$^{6-12)}$ However, the correlation of these conformational characteristics with reactivity of oligonucleotides, if any, have been much less investigated.^{13} The present investigation has been initiated with the aim that the study of non-enzymatic transphosphorylation of certain natural substrates should provide further information for elucidating the mechanism of ribonuclease-catalyzed reactions of these substrates. The annexed scheme for diadenylic acid, a typical dinucleotide, shows the reactions and equilibrium involved in neutral solution, where $(ApAp)_s$ and $(ApAp)_u$ denote "stacked"and "unstacked" species of ApAp, and the portions inside the square bracket exist in dynamic equilibrium [for ApA, $\Delta H^\circ_{\mathrm{unstacking}}$ 5.3 kcal/mole and $\Delta S^\circ_{\mathrm{unstacking}}$ 20 cal/deg.mole[8)]].

$$\left[\begin{array}{c}(\mathrm{ApAp})_s\\ \rightleftharpoons\\ (\mathrm{ApAp})_u\end{array}\right] \xrightarrow[\text{transphosphorylation}]{k} \mathrm{AMP} + \text{Ap-cyclic} \xrightarrow[\text{hydrolysis}]{H_2O} \mathrm{AMP}$$

$$k = k_s[(\mathrm{ApAp})_s] + k_u[(\mathrm{ApAp})_u]$$

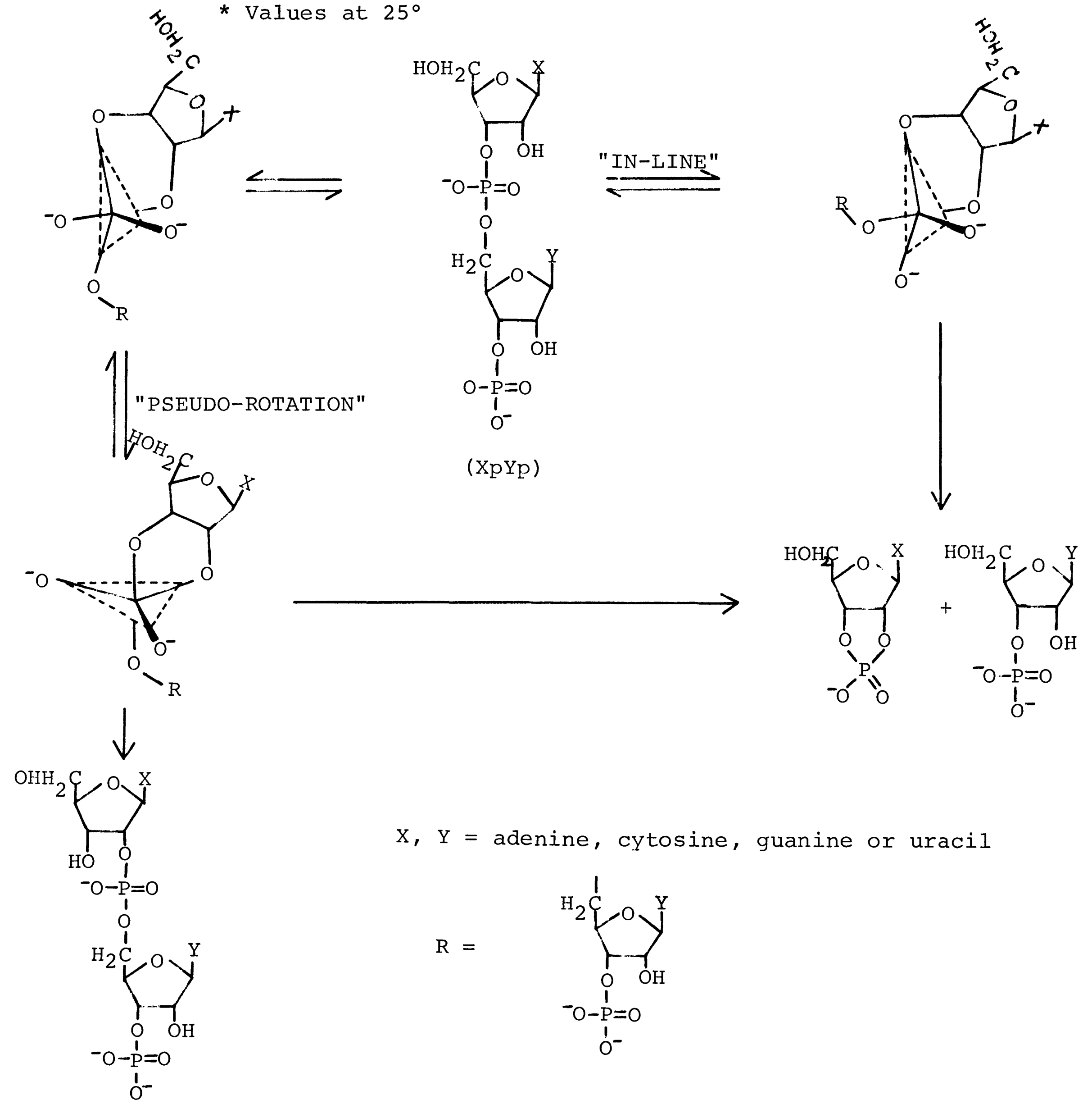
Diribonucleotides used in this study were prepared by a partial hydrolysis of RNA in 0.2M-NaOH followed by column-chromatographic separations with DEAE-Sephadex A-25 and Dowex 1-x2 anion exchangers and purified as previously reported.[14)] On addition of salt-free ApAp(3') and other dinucleotides to 0.2M-NaOH solution, time-dependent changes of the optical properties typical of a single-stranded helical conformation were observed. At 25°C dinucleotides used in this study still have an appreciable fraction of bases in a stacked conformation,[7)] so that, using an auto-recording ORD/UV-5 spectropolarimeter, changes in optical rotation at a selected wavelength with time could be recorded continuously after preparing the solution for reaction. The optical rotation changed followed first-order rate equation, i.e., the plots of $\log[([\phi]_t - [\phi]_\infty)/[\phi]_\infty]$ vs time are good straight lines, and experimental values for the rate constant, k(in sec^{-1}), obtained from these measurements at 25° are: UpGp, 6.47×10^{-5}; CpGp, 3.87×10^{-5}; GpUp, 3.03×10^{-5}; ApUp, 2.09×10^{-5}; ApGp, 2.01×10^{-5}; ApCp, 1.90×10^{-5}; ApAp, 1.53×10^{-5}. The present results again confirmed the previous observation that RNase A-susceptible sequences are more quickly hydrolyzed in alkali than are RNase A-resistant bonds, viz., PyPu and PyPy $>$ PuPu and PuPy linkages.[15)] Furthermore, in a series of adenylyl dimers, ApXp, a small but definite variation in rate was noticed and the stacking interaction tendency of these dimers as measured by the difference ORD, $[\phi]_{\mathrm{dimer}} - \sum[\phi]_{\mathrm{monomer}}$, is in agreement with the above reactivity order, indicating that parallel bases-base stacking interaction is indeed responsible for the reactivity defined in this study. Incidentally, it should be noted that a highly hydrophobic dinucleotide sequence found in *E. coli* ribosomal RNA, N^6,N^6-dimethyl adenylyl-(3'-5')-N^6,N^6-dimethyl adenosine 3'-phosphate, is known to show strong resistance to alkaline hydrolysis.[16,17)]

Thermodynamic parameters for the transphosphorylation have been estimated from the dependence of rate on temperature and are listed in Table I. A striking deviation from Arrhenius plots has been observed in the case of ApAp(3'). Non-linear Arrhenius plots can be explained if the heat capacities are considerably different

Table I. Thermodynamic parameters

Dinucleotide		$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)
ApAp(3')		20.6*	-11.4*
ApCp(3')		19.0	-16.4
ApGp(3')		18.7	-17.2
ApUp(3')		18.6	-17.4
GpUp(3')		17.4	-20.9
UpGp(3')		16.0	-24.0
CpGp(3')		18.4	-17.1
ApAp(3')	at 60°	17.4	-21.6
	at 0.5°	22.3	- 7.9

* Values at 25°



X, Y = adenine, cytosine, guanine or uracil

Figure. Possible routes for the transphosphorylation and breakdown of a dinucleotide.

in the reactant and transition state for the k_s route in the transphosphorylation of ApAp, i.e., $\Delta Cp^{\ddagger} \ll 0$ for k_s route, and in accord with this the energy and entropy of activation were found to decrease as the temperature was raised. A more detailed account will be reported elsewhere.18) Those having one or two protolytic bases (uridylyl or guanylyl residue) in XpYp appear to contribute to a larger negative $\Delta S^{\ddagger}$ value in the reaction, and thus the greater stability of the transition state for UpGp can be attained at the expense of $\Delta S^{\ddagger}$, which becomes more negative. However, more extensive studies are necessary in order to obtain more precise correlation between the activation parameters and the structure of dinucleotides.

Finally, the failure to detect the formation of the (2'-5')-phosphodiester isomer of a dinucleotide, when combined with the "preference rule"19,20) defined by Westheimer, may be taken as supporting evidence for the suggestion that the transphosphorylation of a dinucleotide in alkaline conditions proceeds through simple intramolecular S_N2P or "in-line" mechanism rather than the "pseudo-rotation" mechanism as depicted in Figure.

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