Hydrolyses of *p*-Nitrophenyl(Oligodeoxyribonucleotide Succinate)s by Oligodeoxyribonucleotide *N*-Acetylhistidates on Polycytidylic Acid

Takeo SHIMIDZU* and Robert L. LETSINGER**

* *Department of Hydrocarbon Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606*

** *Department of Chemistry, Northwestern University, Evanston, Illinois, U.S.A.*

(Received February 5, 1973)

The hydrolyses of *p*-nitrophenyl(oligonucleotide succinate)s, in which the oligonucleotides are *d*GpG, *d*GpT, *d*GpC, *d*GpA, and *d*GpGpG, by oligonucleotide *N*-acetylhistidates, in which the oligonucleotides are *d*GpG, *d*GpT, *d*GpC, *d*GpA, and *d*GpGpG, or by *d*GpG nicotinate were carried on polycytidylic acid (Poly C). While the hydrolysis did not occur at lower concentrations of them, it did take place at moderate concentrations, in the presence of Poly C. From the kinetics of the hydrolysis, it was concluded that the interaction between those oligonucleotide derivatives and Poly C was homogeneous at lower concentrations of them and heterogeneous at higher concentrations. The change in the viscosity of the reaction system was correlated with the kinetical results. The heterogeneity in the interaction can be explained as a contiguous stacking of the oligonucleotide derivatives on Poly C. The tendency of the contiguous stacking was great in *d*GpGpG *N*-acetylhistidate. The rate of the hydrolysis of Poly C was higher than that without Poly C. The order in magnitude of the interaction between the oligonucleotide derivatives and Poly C was *d*GpGpG-derivative>*d*GpGp-derivative>*d*GpC-derivative>*d*GpA-derivative>*d*GpT-derivative.

For the study of a chemical reaction on a macromolecule, it is very important to make clear the features of the interaction of a reactant onto the macromolecule. An interaction between polynucleotide and oligonucleotide will play an important role in the condensation of the oligonucleotide on the polynucleotide.

In continuation of an earlier study,[1] the present paper will report on the hydrolyses of *p*-nitrophenyl (oligodeoxyribonucleotide succinate)s, such as *p*-nitrophenyl (deoxyguanylyldeoxyguanosine succinate) (NPS-*d*GpG), -(deoxyguanylylthymidine succinate) (NPS-*d*GpT), -(deoxyguanylyldeoxycytidine succinate) (NPS-*d*GpC), -(deoxyguanylyldeoxyadenosine succinate) (NPS-*d*GpA), and -(deoxyguanylyldeoxyguanylyldeoxyguanosine succinate) (NPS-*d*GpGpG), by oligodeoxyribonucleotide *N*-acetylhistidates, such as deoxyguanylyldeoxyguanosine *N*-acetylhistidate (AH-*d*GpG), deoxyguanylylthymidine- (AH-*d*GpT), deoxyguanylyldeoxycytidine- (AH-*d*GpC), deoxyguanylyldeoxyadenosine- (AH-*d*GpA), and deoxyguanylyldeoxyguanylyldeoxyguanosine (AH-*d*GpGpG), and by deoxyguanylyldeoxyguanosine nicotinate (N-*d*GpG), on polycytidylic acid (Poly C). The hydrolysis of *p*-nitrophenyl(oligodeoxyribonucleotide succinate) proceeds by means of a nucleophilic reaction of an imidazolyl or nicotinyl group present in the latter derivatives. Though the substances used in the present work are not pure and homogeneous, they can still be used in substantially clarifying the features of the interaction of those reactants onto the macromolecule, Poly C.

The interaction between nucleotides is specific and selective, and its magnitude depends on the degree of polymerization of the nucleotides, the concentration of the nucleotides, the ionic strength of the solution, *etc.*[2] Even though guanosine is most liable to stack, no hypochromicity was, in general, observed in the cases of the guanosine dimer and Poly C at which concentrations were of the order of 10^{-4} M.[3] In the case of the enzymatic polymerization of oligonucleotide by DNA-dependent RNA polymerase, the effective degree of polymerization of the oligonucleotide was elucidated to be 6—7.[4] Nevertheless, a complex formation between Poly C and deoxyguanosine-5′-phosphate was suggested by the infrared spectrum under conditions of a higher concentration and a lower temperature.[5] Further, base-base interactions were observed in the dinucleotide model by means of studying the emission spectrum,[6] and in dinucleotide by means of studying the proton magnetic resonance spectrum.[7]

While the interaction between polynucleotide, Poly C, and the oligonucleotide derivatives used is complex, a characteristic mode of interaction is deduced in the present study of the hydrolysis.

Results and Discussion

From the mixing curves for Poly C and NPS-*d*GpG and for Poly C and AH-*d*GpG at the total nucleotide concentration of 7×10^{-4} M, the maximal hypochromicities which occurred at the minimal points were found to be about 1.0% and 2.5% respectively. The larger hypochromicity of the latter might be ascribed to the interaction between the histidyl moiety and the nucleotidic base.

Fig. 1 shows the hydrolysis of NPS-*d*GpG by AH-*d*GpG with Poly C and without Poly C. The rate[8] of the hydrolysis was negligible when the ratio of NPS-

1) T. Shimidzu and R. L. Letsinger, This Bulletin, **44**, 584 (1971).

2) R. Naylor and P. T. Gilham, *Biochemistry*, **5**, 2722 (1966).

3) M. N. Lipsett, *J. Biol. Chem.*, **239**, 1256 (1964).

4) N. K. Gupta, E. Ohtsuka, H. Weber, S. H. Chang, and H. C. Khorana, *Proc. Natl. Acad. Sci. U. S.*, **60**, 285 (1968).

5) F. B. Howard, J. Fraser, M. N. Lipsett, and H. T. Miles, *Biochem. Biophys. Res. Commun.*, **17**, 93 (1964).

6) D. T. Browne, J. Eisinger, and N. J. Leonard, *J. Amer. Chem. Soc.*, **90**, 7302 (1968).

7) C. C. McDonald and W. D. Phillips, *ibid.*, **91**, 1513 (1969).

8) The rate, R, was defined as the increase of *p*-nitrophenol concentration per unit time and per concentration of histidyl or nicotinyl group, at the initial stage of the hydrolysis; $R=[p\text{-nitrophenol}]/([N\text{-acetylhistidine in AH-derivative}]\times t)$ or $R=[p\text{-nitrophenol}]/([\text{nicotinic acid in } N\text{-derivative}]\times t)$.

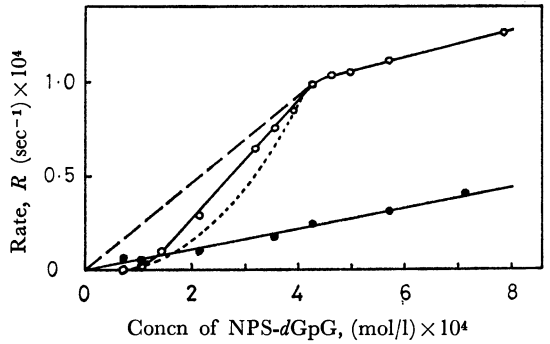

Fig. 1. The total rate of hydrolysis of NPS-*d*GpG by AH-*d*GpG *vs.* their concentration.
Solution; EtOH: H_2O=1 : 100 (0.01M $MgCl_2$, 0.0001M $Mg(OAc)_2$, buffered at a pH being 7.9 with tris(hydroxymethyl)aminomethane; Temperature; 5°C.
○, presence of Poly C (0.35×10^{-3} mol/l).
●, absence of Poly C.

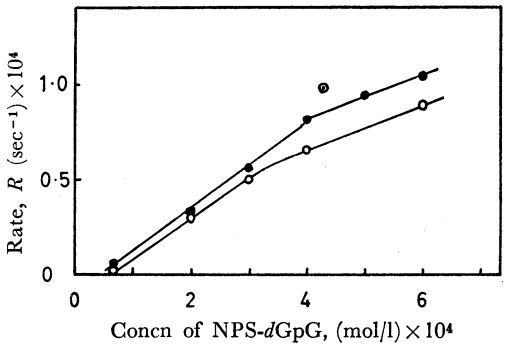

Fig. 2. The total rate of hydrolysis of NPS-*d*GpG by AH-*d*GpG at a constant concentration of AH-*d*GpG.
Conditions are the same in Fig. 1.
○; [AH-*d*GpG]=2.0×10^{-4} mol/l
●; [AH-*d*GpG]=3.0×10^{-4} mol/l
⊙; [AH-*d*GpG]=4.3×10^{-4} mol/l (taken from Fig. 1)

*d*GpG and AH-*d*GpG to Poly C was less than 0.2, but it increased linearly when the ratio exceeded 0.3. When the ratio was over 1.3 the rate of increase of the hydrolysis with Poly C was parallel to that without Poly C. On the other hand, the hydrolysis rate without Poly C was proportional to the amount of the NPS-*d*GpG and AH-*d*GpG, and the line passed through the point of origin.

From the fact that the hydrolysis of the reactants in the presence of Poly C was not observed at lower concentrations, it can be concluded that the interaction between dinucleotide derivatives and Poly C is statistically homogeneous, so that the distance between the two dinucleotide derivatives is too long to cause the hydrolysis reaction. If the two reactants are stacked on Poly C so as to be contiguous with each other, the hydrolysis reaction takes place and the hydrolysis rate can be shown by the broken line[9] in Fig. 1. From the other fact that the hydrolysis rate increased linearly with the amount of the dinucleotide derivatives in the ratio range between 0.3 and 1.3, it can be concluded that, in this range, the derivative is stacked on Poly C contiguously to the derivatives which had previously been stacked on it. If the stacking is homogeneous in this range, the hydrolysis rate can be presented as by the dotted line[10] in Fig. 1. From the other fact that the hydrolysis rate at higher concentrations of the derivatives was almost parallel to that without Poly C, it can be concluded that the stacking of the derivatives on Poly C is saturated in this range and that an excess of them reacts freely with the Poly C.

The difference between the hydrolysis rates with and without Poly C, $R_{polyC}-R_0$, indicates the hydrolysis rate on Poly C. The hydrolysis rate on Poly C increased linearly with the concentration of the reactants, the dinucleotide derivatives, in the ratio of 0.3 to 1.3.

Fig. 2 shows the hydrolysis rate when the concentration of the catalyst, AH-*d*GpG, was a constant. The profile of the increase in the rate with respect to the concentration of the substrate, NPS-*d*GpG, is almost the same as that of Fig. 1 at lower concentrations. The saturation points are different with each concentration of AH-*d*GpG used. They increase with the concentration of AH-*d*GpG. This shows that the reaction on Poly C takes place where the substrate is stacked contiguously with the catalyst. The lowest concentration at which the reaction takes place decreases with the concentrations of the catalyst and the substrate.

The observations in Figs. 1 and 2 support the above-stated conclusions. It can be considered that Poly C does not take a helical configuration under these reaction conditions. Therefore, the following plausible explanation may be offered for this finding: The reactants which were stacked remotely on Poly C at their lower concentrations might not react, while the reactants which were stacked contiguously on Poly C at a moderate concentration could react. At a higher concentration, with a ratio exceeding 1.3, the additional reactants react freely from Poly C.

To estimate the hydrolysis rate on Poly C, let us define the rate as;

$$k_{eff} = (R_{polyC}-R_0)/(C-C_i)$$

where C is the concentration of the reactant, NPS-*d*GpG, and where C_i is the concentration which is the extrapolation of the linearly-increasing part of the rate *vs.* the concentration of NPS-*d*GpG in Fig. 1 to the intercept, or the lowest concentration of which the hydrolysis takes place. The rate constants of the hydrolyses, k_{eff}'s, on Poly C were substantially constants, which were estimated to be $(2.46\pm0.10)\times10^{-1}$ mol^{-1} sec^{-1} at the [NPS-*d*GpG]/[Poly C] ratios exceeding the threshold values respectively. The constancy of the

9) When two reactants are stacked on Poly C contiguously, the hydrolysis rate should be proportional to the concentration of NPS-*d*GpG. So that, the rate is presented as a straight line which passes the origin.

10) When two reactants are stacked on Poly C at random, the average probability of contiguous stacking of those reactants is presented as;

$\bar{P}_c \cong 2n/(n_0-n)$ $\quad (n<n_0/3)$

where n_0 denotes the total contiguous stacking site on the Poly C and n does the number of stacked reactants. The concentration of the reactant (C) is almost proportional to n and the reaction rate is also proportional to $\bar{P}_c$, so the rate is expressed as;

the rate$\cong 2\alpha C/(n_0-\alpha C)$ $\quad (\alpha C \leqq n_0/3)$

where α denotes the stacking factor of the reactant. When $\alpha C > n_0/3$, a proportionality between the rate and the concentration of the reactant will be given.

TABLE 1. RATES OF HYDROLYSES ON AND WITHOUT POLY C

Run	Derivatives of dinucleotide		C_1	Relative rate of hydrolysis[a] ×10	
	Catalyst	Substrate		on Poly C	without Poly C
1	AH-*d*GpG (0.9)	NPS-*d*GpG (1.2)	1.05	2.06/1.05=2.34	0.54/1.05=0.52
2	N-*d*GpG (1.7)	NPS-*d*GpG (1.2)	1.05	0.40/1.45=0.28	0.20/1.45=0.14
3	AH-*d*GpT (1.1)	NPS-*d*GpT (1.4)	0		0.50/1.25=1.25
4	AH-*d*GpC (0.9)	NPS-*d*GpC (1.2)	0.7		0.64/1.05=0.60
5	AH-*d*GpA (0.9)	NPS-*d*GpA (1.1)	>0 ($\times 10^{-4}$ M)		0.48/1.00=0.48 ($1\ mol^{-1}\ sec^{-1}$)

Poly C; 0.35×10^{-3} mol/l. ( ) Shows the degree of esterification. a) The relative rate was obtained by dividing the rate by arithmetic mean values of the esterifications of the substrate and the catalyst.

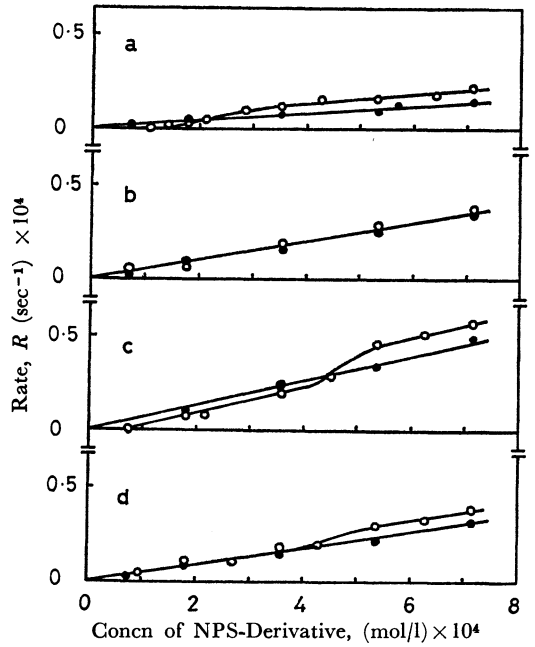

Fig. 3. Hydrolyses of dinucleotide derivatives.
a; hydrolysis of NPS-*d*GpG by N-*d*GpG
b; hydrolysis of NPS-*d*GpT by AH-*d*GpT
c; hydrolysis of NPS-*d*GpC by AH-*d*GpC
d; hydrolysis of NPS-*d*GpA by AH-*d*GpA
○, presence of Poly C (0.35×10^{-3} mol/l)
●, absence of Poly C
The other conditions are the same as in Fig. 1.

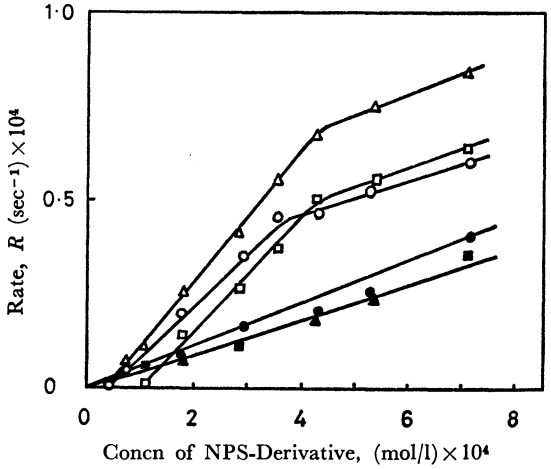

Fig. 4. Hydrolyses of NPS-deoxyguanosine oligomers by AH-deoxyguanosine oligomers.
○: NPS-*d*GpGpG, AH-*d*GpGpG
△: NPS-*d*GpG, AH-*d*GpGpG
□: NPS-*d*GpGpG, AH-*d*GpG
} in the presence of 0.35×10^{-3} mol/l of Poly C.
●: NPS-*d*GpGpG, AH-*d*GpGpG
▲: NPS-*d*GpG, AH-*d*GpGpG
■: NPS-*d*GpGpG, AH-*d*GpG
} in the absence of Poly C.
The other conditions are the same as in Fig. 1.

rate supports the above-mentioned explanation that the reactants stacked on Poly C react contiguously with each other in this range. In other words, the stacking of the reactants on Poly C takes place contiguously in the range of the [NPS-*d*GpG]/[Poly C] ratio exceeding the threshold value. From the hydrolysis rate without Poly C, which is shown in Fig. 1, the rate of the hydrolysis except on Poly C, k_0, is found to be $(0.54\pm0.1)\times10^{-1}\ l\ mol^{-1}\ sec.^{-1}$

Figure 3 shows the hydrolysis of dinucleotide derivatives with and without Poly C. In the case of the hydrolysis of NPS-*d*GpT by AH-*d*GpT, there was no evidence of the interaction with Poly C. In the cases of the hydrolyses of NPS-*d*GpC by AH-*d*GpC, of NPS-*d*GpA by AH-*d*GpA, and of NPS-*d*GpG by N-*d*GpG, similar phenomena were observed. The hydrolyses did not take place at lower concentrations of the oligonucleotide derivatives, and they were parallel to the rate without Poly C at higher concentrations. At moderate concentrations, the hydrolyses increased at a higher rate, similar to the case of the hydrolysis of NPS-*d*GpG by AH-*d*GpG.

To estimate the rate of the hydrolysis on Poly C, k_{eff}, and that without Poly C, k_0, the same calculations as have been described above have been made for each case. The results are tabulated in Table 1. Although the hydrolysis rate of NPS-*d*GpG by AH-*d*GpG on Poly C was 4 times faster than that without Poly C, the hydrolysis rate of NPS-*d*GpG by N-*d*GpG on Poly C was 2 times faster than that without Poly C. This difference might be due to different distances between the catalytic nucleophilic atoms and the stacking points of these nucleotidic materials. The fact that the concentrations at which the hydrolysis took place were substantially identical in both hydrolyses shows that the modes of the interactions of AH-*d*GpG and N-*d*GpG with Poly C are almost identical with that of AH-*d*GpG and NPS-*d*GpG with Poly C. Though it is not possible to quantify the interaction between dinucleotides and Poly C, it can be seen from the values of C_1's that its order is *d*GpG>*d*GpC>*d*GpA>*d*GpT.

Fig. 4 shows the hydrolyses of NPS-*d*GpGpG by AH-*d*GpGpG, of NPS-*d*GpG by AH-*d*GpGpG, and of NPS-

Table 2. Rates of hydrolyses of oligoguanosine derivatives on and without poly C

Run	Derivatives of oligoguanosine		C_i	Relative rates of hydrolysis[a] ×10	
	Catalyst	Substrate		on Poly C	without Poly C
1	AH-*d*GpG (0.9)	NPS-*d*GpG (1.2)	1.0(5)	2.34	0.52
2	AH-*d*GpGpG(0.9)	NPS-*d*GpGpG(1.0)	0.3(5)	0.93	0.57
3	AH-*d*GpGpG(0.9)	NPS-*d*GpG (1.0)	0.3(5)	1.34	0.46
4	AH-*d*GpG (0.9)	NPS-*d*GpGpG(1.0)	0.9	1.09	0.48
			(×10^{-4} M)	(1 mol^{-1} sec^{-1})	

a) Notes are the sameas in Table 1.

Table 3. Reduced viscosities of several reaction systems

Reaction system	η_{sp}/c
0.35×10^{-3} M Poly C	1.15(6)
0.35×10^{-3} M Poly C +0.20×10^{-3} M NPS-*d*GpG & AH-*d*GpG	1.15(6)
0.35×10^{-3} M Poly C +0.40×10^{-3} M NPS-*d*GpG & AH-*d*GpG	1.15(6)
0.35×10^{-3} M Poly C +0.50×10^{-3} M NPS-*d*GpG & AH-*d*GpG	1.11(2)
0.35×10^{-3} M Poly C +0.60×10^{-3} M NPS-*d*GpG & AH-*d*GpG	1.11(2)
0.50×10^{-3} M NPS-*d*GpG & AH-*d*GpG	
0.35×10^{-3} M Poly G	1.31(6)
0.35×10^{-3} M Poly C+0.35×10^{-3} M Poly G	2.34(7)
	1.10(5)[a]

c: g-solute/100 ml relating to Poly C (=0.0106)
5±0.05°C; 0.01 M $MgCl_2$, 0.0001 M $Mg(OAc)_2$, EtOH-H_2O =1 : 100
a) Total concentration of Poly C and Poly G was used as*c* (=0.0225) in the calculation.

*d*GpGpG by AH-*d*GpG. Profiles of hydrolysis similar to Figs. 1 and 4 were observed.

The rates are tabulated in Table 2. From the hydrolyses of NPS-*d*GpG by AH-*d*GpG and of NPS-*d*GpGpG by AH-*d*GpGpG, it is very tempting to suppose that the distance between the substrates and the catalysts plays a role in the hydrolyses. Although the two relative rates of hydrolyses without Poly C were similar, the rates on Poly C were different. The longer distance was not preferable to the hydrolysis in spite of the tightly stacking on Poly C. The exhibition of very little increment in the hydrolysis of the latter might be due to the stacking of AH-*d*GpGpG and NPS-*d*GpGpG with each other. Furthermore, a directionality in the interaction might be considered with the difference between the rates of the hydrolyses of NPS-*d*GpG by AH-*d*GpGpG and that of NPS-*d*GpGpG by AH-*d*GpG. From the C_i's which are concentrations of the oligonucleotide derivatives when the hydrolysis took place, we learn that the mode of interaction of AH-*d*GpGpG with Poly C was rather favorable to the contiguous stacking.

The results of the reduced viscosity measurements of several reaction systems are tabulated in Table 3. While the reduced viscosity, η_{sp}/c, relating to Poly C did not change when relatively small amounts of NPS-*d*GpG and AH-*d*GpG were present in the system, the reduced viscosity decreased when amounts of NPS-*d*GpG and AH-*d*GpG of more than 0.50×10^{-3} M were present. The viscosity of the system of NPS-*d*GpG and AH-*d*GpG was negligible, of course. On the other hand, when an added Poly G existed in the Poly C solution, the reduced viscosity relating to Poly C obviously increased to 2.34(7). This increase in the viscosity was due to the added Poly G. To eliminate the effect of the viscosity of Poly G, we employed the total concentrations of those two polymeric materials, Poly C and Poly G, as the reference concentration, *c*; then, the reduced viscosity was estimated to be 1.10(5). This value was less than the arithmetic mean value of the reduced viscosities of Poly C and Poly G. This can be explained as the formation of a double strand of Poly C and Poly G by reference to the results of Fresco and Doty.[11] Using Poly A, they had obtained a lower viscosity under the condition of an interrupted helix and a higher viscosity under the condition of a random coil. Therefore, the decrease in the reduced viscosity of Poly C in the presence of a considerable amount of NPS-*d*GpG and AH-*d*GpG can be explained as a partial conformational change of Poly C at the interacting location where the stacking of those dinucleotide derivatives with Poly C took place.

Those results are compatible with the conclusions that the stacking of the dinucleotide derivatives with Poly C takes place statistically homogeneous at the initial stage and that afterwards the stacking occurs at the site contiguously to the pre-stacked one. Such a phenomenon may be caused by a conformational change in Poly C by the stacking of the oligonucleotide derivatives. This conclusion is an interesting concept compared with that of the homogeneous stacking previously believed.

Experimental

The hydrolyses reactions were carried out using a 1 : 100 ethanol-water solution containing 0.01 M of $MgCl_2$ and 0.0001 M of $Mg(OAc)_2$,[12] the pH of which was made up to 7.9 by tris-(hydroxymethyl)aminomethane.

The Poly C solution was made up to 0.7×10^{-3} M using the above buffer solution. Both solutions of dinucleotide and trinucleotide derivatives in a certain concentration were prepared fresh each time using the above buffer solution.

11) J. R. Fresco and P. Doty, *J. Amer. Chem. Soc.*, **79**, 3928 (1957).
12) Generally, existence of Mg^{2+} assists the complex formation of Poly C-Poly G, so that such magnesium salts were used in the present study. The existence of Mg^{2+} did not affect the absorbancy of Poly C in UV spectrum.

The experiments were carried out by mixing 2 ml of the Poly C solution, 1 ml of the substrate derivative of the *p*-nitrophenyl succinate solution, and 1 ml of the catalyst derivative of the *N*-acetylhistidate or nicotinates solution at 5 °C. The absolute amounts of the substrate and the catalyst were the same in all the experiments. The rates of the hydrolyses were measured by the increase in absorbance at 400 nm. In the case of the absence of Poly C, 2 ml of the above buffer solution instead a similar portion of the Poly C solution was used.

By analysing the reacted material in a paper chromatograph, it was proved that the hydrolyzed materials did not contain *p*-nitrophenylsuccinic acid. It can be said that the hydrolyses took place predominantly at the position between *p*-nitrophenyl and succinyl moieties.

The increase in absorbance can be treated as linear with the reaction time at lower conversions. The reaction followed the kinetics of the first-order with respect to the NPS-*d*GpG to the extent of 45% of the conversion. This shows that there might be no product inhibition in the reaction.

The viscosity was measured at 5±0.05 °C using a Ubbelohde-type viscometer.

Polycytidylic Acid (Poly C) and Polyguanylic Acid (Poly G). Poly C and Poly G were purchased from the Miles Chemical Co.; their sedimentation constants were 4.3 and 2.9 respectively.

p-*Nitrophenyl (deoxyguanylyldeoxyguanosine succinate) (NPS-*d*GpG).* In anhydrous pyridine, 40 mg of *N*-di-*p*-methoxytrityldeoxyguanylyl-*N*-di-*p*-methoxytrityldeoxyguanosine,[13] (DMTr)*d*G(DMTr)*d*G, and 20 mg of succinic anhydride was subjected to a reaction for 14 hr at room temperature; then we added 25 mg of *p*-nitrophenol and 40 mg of dicyclohexylcarbodiimide. After 14 hr, 10 ml of an aqueous solution of KH_2PO_4 was added. Then 90 ml of ethanol was added, and the resultant mixture was filtered. A filtrate was concentrated, and 40% acetic acid was added along with a small quantity of pyridine. After 5 hr, the solution was separated by centrifugation. The isolation of NPS-*d*GpG was achieved chromatographically in 70% ethanol using an anion-exchange resin, IR-45. The resulting NPS-*d*GpG contained 1.2 *p*-nitrophenyl groups per molecule, as indicated by the ultraviolet spectra before and after alkaline hydrolyses, which were achieved with 1 M NaOH. It is probable that the nitrophenylsuccinyl group is bonded predominantly to the 5′-O position of the nucleotide. The resulting NPS-*d*GpG did not obtain free carboxylic acid, since the present column separation showed that the resulting NPS-*d*GpG was found in one elution band, unlike the case of a free carboxylic acid.

p-*Nitrophenyl(deoxyguanylylthymidine succinate) (NPS-*d*GpT),* p-*Nitrophenyl(deoxyguanylyldeoxycytidine succinate) (NPS-*d*GpC),* p-*Nitrophenyl(deoxyguanylyldeoxyadenosine succinate) (NPS-*d*GpA),* and p-*Nitrophenyl(deoxyguanylyldeoxyguanylyldeoxyguanosine succinate) (NPS-*d*GpGpG).* The procedures to synthetize these materials were the same as that for NPS-*d*GpG, but using *N*-di-*p*-methoxytrityldeoxyguanylylthymidine, (DMTr)*d*GT, in lieu of (DMTr)*d*G(DMTr)*d*G, using *N*-di-*p*-methoxytrityldeoxyguanylyl-*N*-di-*p*-methoxytrityldeoxycytidine, (DMTr)*d*G(DMTr)*d*C, in lieu of (DMTr)*d*G(DMTr)*d*G, using *N*-di-*p*-methoxytrityldeoxyguanylyl-*N*-di-*p*-methoxytrityldeoxyadenosine, (DMTr)*d*G(DMTr)*d*A, in lieu of (DMTr)*d*G(DMTr)*d*G, and using *N*-di-*p*-methoxytrityldeoxyguanylyl-*N*-di-*p*-methoxytrityldeoxyguanylyl-*N*-di-*p*-methoxytrityldeoxyguanosine,[14] (DMTr)*d*G(DMTr)*d*G(DMTr)*d*G, in lieu of (DMTr)*d*G(DMTr)*d*G.

Deoxyguanylyldeoxygaunosine N-*acetylhistidate (AH-*d*GpG).* In anhydrous pyridine, 30 mg of *N*-di-*p*-methoxytrityldexoyguanylyl-*N*-di-*p*-methoxytrityldeoxyguanosine, (DMTr)*d*G(DMTr)*d*G, 30 mg of *N*-acetylhistidine, and 50 mg of dicyclohexylcarbodiimide were reacted for 4 days at room temperature. Then 30 ml of a KH_2PO_4 aqueous solution was added. An 50 ml of ethanol was added and filtered. Into the filtrate, the same amount of acetic acid was poured. After 5 hr the supernatant was separated by centrifugation. The isolation of AH-*d*GpG was achieved chromatographically in 70% ethanol using the anion-exchange resin. The resulting AH-*d*GpG contained 0.9 imidazolyl group per molecule, as indicated by the ultraviolet spectra in an acidic solution, in which the nucleotide had been removed by means of an ion-exchange column, before and after alkaline hydrolyses. In this case, it is probable that the histidyl group is bonded predominantly to the 5′-O position of the nucleotide.

Deoxyguanylylthymidine N-*acetylhistidate (AH-*d*GpT), Deoxyguanylyldeoxycytidine* N-*acetylhistidate (AH-*d*GpC), Deoxyguanylyldeoxyadenosine* N-*acetylhistidate (AH-*d*GpA), and Deoxyguanylyldeoxyguanylyldeoxyguanosine* N-*acetylhistidate (AH-*d*GpGpG).* The procedures to synthetize these materials were the same as that for AH-*d*GpG, but using (DMTr)*d*GT in lieu of (DMTr)*d*G(DMTr)*d*G, using (DMTr)*d*G(DMTr)*d*C in lieu of (DMTr)*d*G(DMTr)*d*G, using (DMTr)*d*G(DMTr)*d*A in lieu of (DMTr)*d*G(DMTr)*d*G, and using (DMTr)*d*G(DMTr)*d*G(DMTr)*d*G in lieu of (DMTr)*d*G(DMTr)*d*G.

*Deoxyguanylyldeoxyguanosine nicotinate (*N-d*GpG).* Thirty mg of (DMTr)*d*G(DMTr)*d*G and 30 mg of nicotinyl chloride were reacted in anhydrous pyridine for 4 hr at room temperature. Two ml of methanol was added to esterify the excess of nicotinyl chloride; then the solution was condensed until it became cloudy, and 30 ml of acetic acid was added. After 4 hr, the isolation of N-*d*GpG was achieved chromatographically in a 50% ethanol aqueous solution, using the anion-exchange resin.

The nitrophenyl, imidazolyl, and nicotinyl contents in the above materials are tabulated in Table 4.

Table 4. Contents of nitronphenyl, imidazolyl, and nicotinyl groups in the esters

	Substrate	Catalyst
	p-Nitrophenyl-succinate	*N*-Acetyl-histidate
*d*GpG	1.2	0.9
*d*GpT	1.4	1.1
*d*GpC	1.2	0.9
*d*GpA	1.1	0.9
*d*GpGpG	1.0	0.9
		Nicotinate
*d*GpG		1.7

13) T. Shimidzu and R. L. Letsinger, *J. Org. Chem.*, **33**, 708 (1968).

14) T. Shimidzu and R. L. Letsinger, This Bulletin, **44**, 1673 (1971).