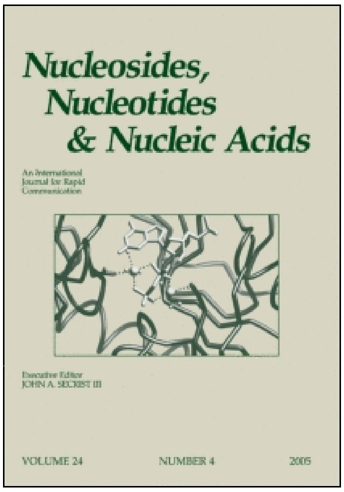

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KINETIC APPROACH FOR DETERMINATION OF AFFINITY PROPERTIES OF REACTIVE OLIGONUCLEOTIDE DERIVATIVES TO COMPLEMENTARY REGIONS IN NUCLEIC ACIDS

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Abstract: The kinetic and thermodynamic parameters of intraduplex DNA modification with 5'-p-azidoperfluorobenzene phosphoramide derivative of oligonucleotide were studied.

Scheme

$$\begin{tikzcd}
 & PZ & \\
PX \arrow[r, "\gamma k_0"] \arrow[d, Leftrightarrow, "K_x"'] & PI \arrow[u, "k_1"] \arrow[r, "k_2'"] \arrow[d, shift left, "k_{-i}"] & PR \arrow[d, Leftrightarrow, "K_r"] \\
P+X \arrow[r, "k_0"] & P+I \arrow[r, "k_2"] \arrow[u, shift left, "k_i"] & P+R
\end{tikzcd}$$

Since 1981 we have used the kinetic study for the determination of association constants of alkylating oligonucleotide derivatives with the complementary sequences of nucleic acids [1,2]. The mechanism of the nucleic acid modification was described by the Scheme where P is nucleic acid target, X is the oligonucleotide derivative attached to alkylating N-methyl-N-(2-chloroethyl)-aminophenyl group, I intermediate ethyleneimmonium cation formed from the reagent in the limiting step, R is the product of reagent consumption in solution, PX, PR and PI are the complexes with the target. If the product R retains the affinity to the target and $K_x = K_r$, the limit modification yield at t →∞ was described by the equation:

$\frac{[PZ]_\infty}{p_o} = 1 - \exp\left(-\frac{\gamma_{ef} K_x x_o}{1 + K_x x_o}\right)$ (1) , where $\gamma_{ef} = \frac{k_1}{k_1 + k_{-i} + k_2'}$ (2) is the efficiency of intracomplex alkylation. It is seen that using the dependence of the limit modification extent on the reagent concentration the association constant K_x can be determined. This approach of K_x determination was used further for the catalitically active oligonucleotide derivative carrying Fe-porphyrin group [3]. More complex situation occurs if the reagent loses partly the affinity to the target during the conversion in solution. In this case the kinetic equation

$$\frac{[PZ]}{p_0} = 1 - \left[\frac{1 + K_x x_o}{1 + K_r x_o + (K_x - K_r) x_o \exp(-k_0 t)}\right]^{\frac{\gamma_{ef} K_x}{K_r - K_x}} \quad (3)$$

contains four unknown parameters: K_x, K_r, k_0 and γ_{ef}. This was found to be the case in the modification by 5'-p-azidoperfluorobenzene phosphoramide derivatives of oligonucleotide d(pCA)$_3$ (reagent **I**). Under irradiation by light of Hg-lamp with wavelenght > 300 nm the p-azidoperfluoro-benzoic acid is converted only to one product as follows from Fig.1, where three isobestic points are observed. Rate constant of photolysis was obtaind and found to be equal to $k_p = (4.05 \pm 0.38) \times 10^{-4}\ \mathrm{s}^{-1}$. If reagent **I** was irradiated the oligonucleotide moiety degraded mainly due to modification of dA-4 and the affinity to the target was lost partly. For the quantitative treatment of the modification data and determination of unknown parameters K_x, K_r, k_0 and γ_{ef} we have used the following approach. The association constant of the reagent I with the target d(pGTGTGA) was determined according Kitz-Wilson equation [4] which describes the dependence of the affinity modification rate on the initial reagent concentration. The product of cross-linking between the reagent I and G-3 residue of the target was identified by PAGE. As a measure of the initial rate we have taken the modification extent at 180 second, when the oligonucleotide moiety of the reagent was not damaged: $[PZ]_t / p_0 = \alpha \cdot \frac{K_x x_0}{1 + K_x x_0}$, (4) where α is coefficient. K_x was found to be $(1.40 \pm 0.24) \times 10^5\ \mathrm{M}^{-1}$. K_r value was determined in the experiments with the preliminary irradiated reagent (irradiation time =7000 s) using the equation

$$[PZ]_\infty / p_0 = 1 - \left[\frac{1 + K_x x_0 + K_r r_0}{1 + K_r (x_0 + r_0)}\right]^{\gamma \frac{K_x}{K_x - K_r}} \qquad (5)$$

where r_0 is the concentration of preliminary irradiated reagent. It was found that $K_r/K_x = (0.28 \pm 0.02)$ and consequently $K_r = (2.49 \pm 0.30) \times 10^4\ \mathrm{M}^{-1}$. Using K_x and K_r values the kinetic curve (Fig.2) of target modification was treated according the equation (3) and values $k_o = (5.31 \pm 0.28) \times 10^{-4}\ \mathrm{s}^{-1}$, $\gamma_{ef} = (1.02 \pm 0.04)$ were found.

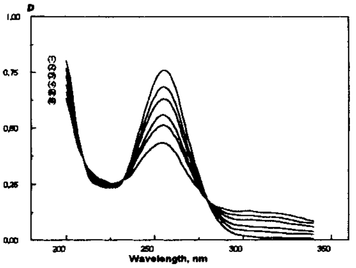

FIG. 1. Photodegradation of p-azidoperfluorobenzoic acid in solution. The irradiation time was 0, 500, 1000, 2000, 3000, 5000 sec. for curves (1-6) respectively (0.16M NaCl, 0.02M Na_2HPO_4, 0.1mM EDTA, pH=7.5 at 20°C).

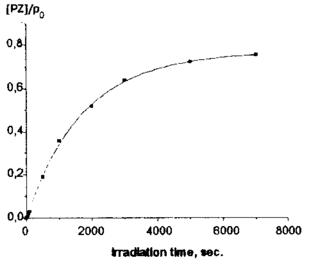

FIG. 2. Kinetics of the target d(pGTGTGA) modification. Concentration $x_0 = 4.9 \cdot 10^{-5}$M, $p_0 = 1 \cdot 10^{-8}$ M.

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