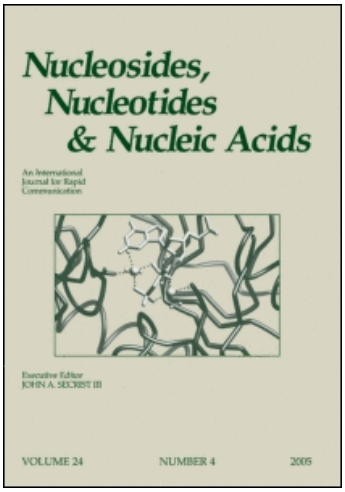

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Poly(L-Lysine)-Graft-Dextran Copolymer Remarkably Promotes Pyrimidine-Motif Triplex Formation at Neutral Ph: Thermodynamic and Kinetic Studies

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POLY(L-LYSINE)-GRAFT-DEXTRAN COPOLYMER REMARKABLY PROMOTES PYRIMIDINE-MOTIF TRIPLEX FORMATION AT NEUTRAL PH: THERMODYNAMIC AND KINETIC STUDIES

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ABSTRACT: The binding constant of a homopyrimidine oligonucleotide with its target duplex for the pyrimidine-motif triplex formation at neutral pH was analyzed and found to be remarkably higher in the presence of the poly (L-lysine)-*graft*-dextran copolymer than that without any triplex stabilizer or in the presence of spermine. The promoting effect mainly results from the considerable increase in the association rate constant.

Extreme instability of pyrimidine-motif triplex DNA at physiological pH severely limits its utility for artificial control of gene expression *in vivo*. Stabilization of the pyrimidine-motif triplex at neutral pH is, therefore, of great importance to improve its therapeutic potential. We have previously shown that poly (L-lysine)-*graft*-dextran (PLL-*g*-Dex) copolymer (1) stabilizes the pyrimidine-motif triplex within the rat α1(I) collagen gene promoter at neutral pH (2). Here, using a 23-mer duplex, (Pur23A•Pyr23T) and a 15-mer homopyrimidine single strand (Pyr15T), we have further extended our study to explore the thermodynamic and kinetic effects of the PLL-*g*-Dex copolymer on the pyrimidine-motif triplex formation at pH 6.8.

Both thermodynamic and kinetic analyses, using isothermal titration calorimetry (ITC) (3, 4) and interaction analysis system (IAsys) (4), respectively, have revealed

TABLE 1: Thermodynamic parameters for the triplex formation between Pyr15T and Pur23A•Pyr23T at 25 °C and pH 6.8 with or without the triplex stabilizer by ITC

triplex stabilizer	Ka(M^{-1})	Ka (relative)	ΔG(kcal mol^{-1})	ΔH(kcal mol^{-1})	ΔS(cal mol^{-1} K^{-1})
None	1.97×10^5	1.0	-7.22	-34.9	-92.7
840 μM spermine	5.15×10^5	2.6	-7.79	-34.1	-88.2
38 μM copolymer	1.89×10^7	95.9	-9.93	-87.9	-262

TABLE 2: Kinetic parameters for the triplex formation between Pyr15T and Pur23A• Pyr23T at 25 °C and pH 6.8 with or without the triplex stabilizer by IAsys

triplex stabilizer	$k$ass(M^{-1} s^{-1})	$k$ass(relative)	$k$dis(s^{-1})	$k$dis(relative)	Ka(M^{-1})	Ka(relative)
None	6.31×10^2	1.0	1.17×10^{-2}	1.0	5.41×10^4	1.0
840 μM spermine	2.29×10^3	3.6	1.06×10^{-2}	0.91	2.15×10^5	4.0
38 μM copolymer	2.88×10^4	45.6	0.78×10^{-2}	0.66	3.69×10^6	68.2

that, in the presence of the copolymer, the binding constant, Ka, of the pyrimidine-motif triplex formation is about 100-times higher than that observed without any triplex stabilizer (TABLEs 1 and 2). In addition, the triplex-stabilizing efficiency of the copolymer is remarkably higher than that of physiological concentration (about 1 mM) of spermine, a putative triplex stabilizer (TABLEs 1 and 2). Kinetic data have also demonstrated that the copolymer-mediated promotion of the triplex formation results from the considerable increase in the association rate constant rather than the decrease in the dissociation rate constant (TABLE 2). Our results certainly support an idea that the PLL-*g*-Dex copolymer could be a key material and may eventually lead progress in therapeutic applications of the antigene strategy *in vivo*.

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