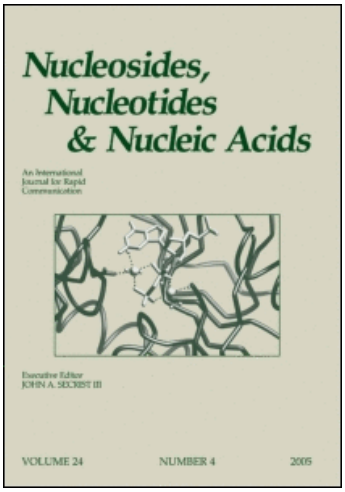

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Relative Effects of Graft Copolymer and Polyamines on Triplex Stabilization Under Physiological Conditions

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RELATIVE EFFECTS OF GRAFT COPOLYMER AND POLYAMINES ON TRIPLEX STABILIZATION UNDER PHYSIOLOGICAL CONDITIONS

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ABSTRACT: Triplex-stabilizing effect of a graft copolymer under physiologically relevant conditions has been evaluated and compared with other polyamines. Here we show that the graft copolymer significantly stabilizes triplex DNAs with amazingly higher efficicacy than that of physiological concentrations of spermine and spermidine.

Triplex formation by sequence-specific interaction of triplex-forming oligonucleotides (TFOs) within a short homopurine–homopyrimidine stretch of native duplex has got a great attention for its possible therapeutic applications to modulate gene functions.[1] However, triplex DNA of pyrimidine motif and purine motif involving unmodified TFO is unstable under physiological conditions due to their pH- and potassium ion (K^+) sensitivity, respectively.[2] We have previously demonstrated that poly(L-lysine)-graft-dextran copolymer (Fig. 1a) significantly increased the thermal stability of the triplex structure with poly(dA)•2poly(dT) [3] and also stabilizes triplex DNAs within a short (30-mer) native duplex from rat α1(I) collagen gene promoter (Fig. 1b) at physiologic pH and K^+ concentrations.[4] Here we have compared the triplex-stabilizing efficiency of the graft copolymer and other polyamines, like spermine and spermidine. We will show that the triplex-stabilizing ability of the copolymer is remarkably higher than that of other polyamines.

We found that purine motif triplex formation without KCl at 0.17 μM Pu-20 slightly differs (8%) in the presence or absence of the copolymer. A drastic decrease in triplex formation is, however, observed with an increase in K^+ concentrations, while the inhibitory effect was amazingly abrogated by adding the copolymer during triplex formation (data not shown)[4]. Of interest is that the copolymer also significantly overcomes pH-dependency in pyrimidine motif triplex formation (Fig. 2). Moreover,

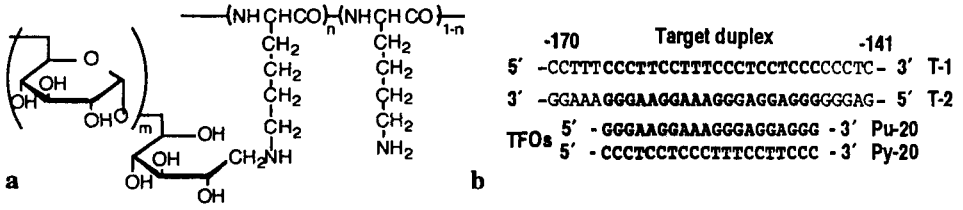

FIG. 1: (a) Structural formula of the copolymer. (b) Oligonucleotides used in this study

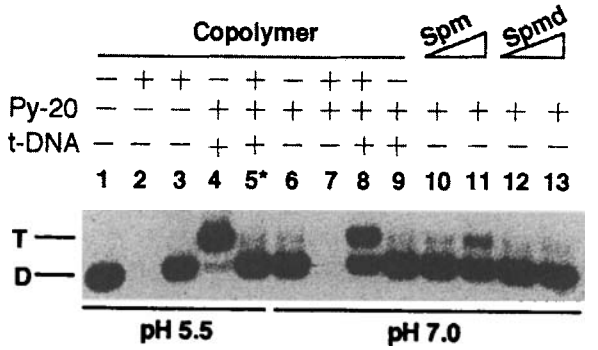

FIG. 2: Comparison of triplex-stabilizing efficiency between the copolymer, spermine (Spm) and spermidine (Spmd). Reaction mixtures were incubated at pH 5.5 (lanes 1–4) or 7 (lanes 6–13) with (+) or without (-) 1.7 μM Py-20, 2.5 μg copolymer, 0.2 mM (lanes 10 &12) and 1 mM (lanes 11 & 13) of Spm and Spmd. After 6 h incubation at 37 °C, 1 μl 500 mM Tris-acetate (pH 5) with (+) or without (-) t-DNA was added in lanes 8–13 and the samples were electrophoresed at pH 5.5 to separate duplex (D) and triplex (T) DNA. In lane 5, reaction was analyzed without incubation.

the triplex-stabilizing efficiency was remarkably higher than that of physiological concentrations of spermine and spermidine (Fig. 2) [4]

It has been reported that the triplex-stabilizing efficiency of polyamines is considerably reduced under physiological conditions due to their competitive replacement by co-existing monovalent cations.[5] Our recent analyses reveal that tremendous increase in association rate constant is the major contribution for copolymer-mediated triplex stabilization (Torigoe, et. at., manuscript in preparation). Since over expression of collagen gene causes liver cirrohsis, triplex stabilization by the copolymer will be a cue for further progress in gene therapy even though its effect *in vivo* is yet unknown.

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