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Ionic and Structural Specificity Effects of Natural and Synthetic Polyamines on the Aggregation and Resolubilization of Single-, Double-, and Trile-Stranded DNA

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DNA condensation, precipitation, and aggregation are related phenomena involving DNA-DNA interactions in the presence of multivalent cations, and studied for their potential implications in DNA packaging in the cell. Recent studies have shown that the condensation/aggregation is a prerequisite for the cellular uptake of DNA for gene therapy applications. To elucidate the ionic and structural factors involved in DNA ggregation, we studied the precipitation and resolubilization of high molecular weight and sonicated calf thymus DNA, two therapeutic oligonucleotides, and poly(dA)・2Poly(dT) triplex DNA in the presence of the tetravalent polyamine spermine using a centrifugation assay, Tm measurements, and CD spectroscopy. The ability of spermine to provoke DNA precipitation was in the following order: triplex DNA > duplex DNA > single-stranded DNA. In contrast, their resolubilization at high polyamine concentrations followed a reverse order. The effective concentration of spermine to precipitate DNA increased with Na^+ in the medium. Tm data indicated the DNA stabilizing effect of spermine even in the resolubilized state. CD spectroscopy revealed a series of sequential conformational alterations of duplex and triplex DNA, with the duplex form regaining the B-DNA conformation at high concentrations (～200 mM) of spermine. The triplex DNA, however, remained in a Ψ-DNA conformation in the resolubilized state. Chemical structural specificity effects were exerted by spermidine and spermine analogues in precipitating and resolubilizing sonicated calf thymus DNA, with N^4-methyl substitution of

ing sonicated calf thymus DNA, with N^4-methyl substitution of spermidine and a heptamethylene separation of the imino groups of spermine having the maximal difference in the precipitating ability of the analogues compared to spermidine and spermine, respectively. Therapeutically important bis(ethyl) substitution reduced the precipitating ability of the analogues compared to spermine. The effect of the cationicity of polyamines was evident with the pentamines being much more efficacious than the tetramines and triamines. These results provide new insights into the mechanism of DNA precipitation by polyamines, and suggest the importance of polyamine structure in developing gene delivery vehicles for therapeutic applications.