Selective Stabilization of Triplex DNA by Poly(ethylene glycols)

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Received July 21, 1995

Interest in the structure and stability of triple-helical forms of nucleic acids has increased in recent years for several reasons. First, the formation of triplex adducts at specific DNA sequences has been proposed as a possible way to inhibit gene expression,[1] or as a means to design new site-specific reagents for the cleavage of genomic DNA.[2] Second, triplex H-form DNA was discovered in certain plasmid sequences, suggesting that triple helices may have a functional biological role.[3] Several studies have appeared describing the thermodynamics of triple-helix formation and the stability of various triplex strand combinations.[4] The stability of the triplex is influenced by the specific base sequences involved, as well as by factors such as pH, temperature, and salt concentration.[4] We report here a rather remarkable effect on the stability of the triplex poly(dT)−poly(dA)−poly(dT) by a series of poly(ethylene glycols), polymers often used as agents for the study of solution crowding effects.[5] Dramatic increases in the melting temperature of the triplex occur with increased concentrations of polymer. These effects are much greater than observed for duplex melting transitions under the same solution conditions.

Poly(dT)−poly(dA)−Poly(dT) triplexes were formed by mixing duplex poly(dA)−poly(dT) with single-stranded poly(dT) such that the number of single-stranded nucleotides was equivalent to the number of base pairs in the duplex, using UV absorbance measurements of the component polynucleotides. The solutions were made up in a buffer consisting of 300 mM NaCl (150 mM NaCl in several experiments), 10 mM sodium cacodylate, and 0.1 mM EDTA, with the pH adjusted to 7.4 (CNE buffer). A stock solution of triplex was annealed by heating to 85 °C and slowly cooling to room temperature before use in the melting experiments. The influence of several high molecular weight poly(ethylene glycols) on triplex and duplex melting was examined. Solutions of Peg 8 (MW = 8000), Peg 3 (MW = 3400), Peg 1 (MW = 1000), Peg 04 (MW = 400), and the monomer, ethylene glycol, obtained from Sigma Chemical, St. Louis, MO, were prepared in CNE buffer, and then stock triplex was diluted into the appropriate glycol solution for melting experiments.

Figure 1 shows representative differential UV melting transition curves (shown as dA/dT) vs temperature) for two of the polyethylene glycol media studied. The lower temperature transitions in the figure are the triplex melts,[4] and the higher temperature transitions are for the duplex, poly(dA)−poly(dT). Notice for the Peg 3 solutions that the T_m for the triplex melt

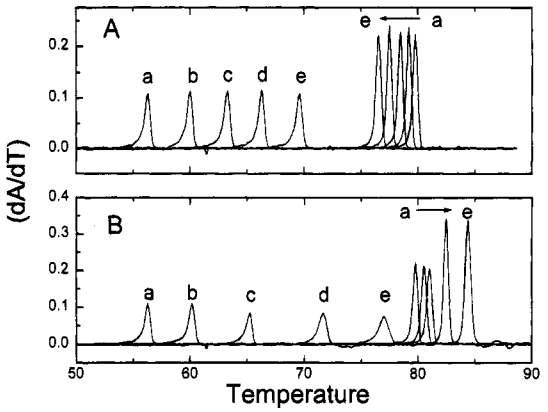

Figure 1. Derivative UV melting curves for triplex, poly(dT)−poly(dA)−poly(dT) in polyethylene glycol 400 (A) and 3400 (B). Percentages (w/v) of added polyethylene glycol are (a) 0%, (b) 4.8%, (c) 9.6%, (d) 14.3%, (e) 19.1%. Note that, in panel A, the high-temperature melting transition of the duplex *decreases* with increasing polyethylene glycol concentration. In panel B, the high-temperature melting transition of the duplex *increases* with increasing polyethylene glycol concentration.

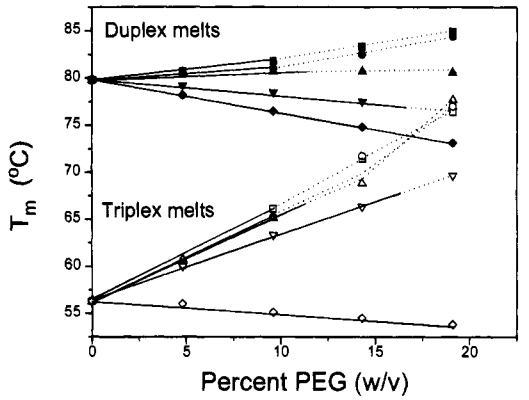

Figure 2. Melting temperatures of duplex (poly(dA)−poly(dT)) and triplex (poly(dT)−poly(dA)−poly(dT)) versus percentage of poly(ethylene glycol) in solution. Peg 8000 (□, ■), Peg 3400 (○, ●), Peg 1000 (△, ▲), Peg 400 (▽, ▼), and ethylene glycol (◇, ◆).

has increased by more than 20 deg from 56 to 77 °C as the Peg 3 concentration increased from 0 to 19%. The duplex melting temperatures increase only slightly over the same range of concentrations. For the smaller polymer, Peg 04, it is interesting that the triplex melt again increases from 56 to almost 70 °C, but the duplex melting temperature actually decreased over the same range of Peg 04 concentrations. The monomer, ethylene glycol, which is not a crowding agent but does affect water activity,[6] decreases duplex melting by about 6 deg, while the triplex only decreases 2.5 deg as glycol concentration increases to 19%. (Primary data not shown.) Figure 2 summarizes the melting temperatures for the triplex and duplex in the various solutions examined.

Melting experiments were also carried out for a triplex formed from the same duplex, poly(dA)−poly(dT), but using as third strand a short, 20-nucleotide oligomer, $(dT)_{20}$. In the media studied, Peg 8 and Peg 3, the variation in T_m was virtually identical with that found for the triplexes formed using the longer poly(dT) (data not shown).

The stability of double and triple helices is strongly dependent upon salt concentration. Additional melting experiments were conducted in CNE buffer containing 150 mM NaCl using a range of concentrations of either Peg 3 or Peg 04 (results shown as supporting information). In the lower salt concentration in

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the absence of Peg, the T_m values of both the triplex and duplex decreased, to 33 and 64.6 °C, respectively. The effect of added Peg 3 or Peg 04 was qualitatively the same as was observed in 300 mM NaCl, namely, a selective stabilization of the triplex. Addition of 20% Peg 3 resulted in a 20 deg (±1.0 deg) increase in the triplex T_m, while the T_m of the duplex was increased by only 5 deg (±1.0 deg). Addition of 20% Peg 04 resulted in a 17.5 deg (±1.0 deg) increase in the triplex T_m, a slightly higher value than was observed in 300 mM NaCl. The key point remains, however, that the triplex is dramatically stabilized by the addition of Peg 04, while the duplex is slightly destabilized (see Figure 2 in supporting information). Triplex stability is a complex function of multiple factors, including salt concentration, water activity, and crowding-agent concentration. Quantitative understanding of the effects of these variables requires mapping of the complete phase diagram. Such detailed studies are in progress. The selective stabilization of triplex DNA by added Peg is qualitatively clear at the two NaCl concentrations reported here.

It is known for duplex poly(dA)−poly(dT) that, in moderate salt concentration and at Peg 8 content around 15% w/v, the condensed ψ form of DNA is found.[7] Since, for the triplex melts at higher concentrations of Peg 8 and Peg 3, the melting transition derivative peak areas were somewhat smaller, circular dichroism (CD) spectra were obtained for most of the solutions on which melting experiments were done to check for the presence of ψ forms of DNA. For all of the 19% solutions except ethylene glycol the CD spectra were markedly different from the triplex spectrum in CNE buffer alone. In these concentrated solutions there are large peaks at 270 and 215 nm, and the general features of the spectrum resemble those seen for the ψ^+ DNA formed from poly(dA)−poly(dT).[7] The triplex in Peg 04 polymer concentrations below 19% showed CD spectra identical with those in CNE buffer, and for Peg 1 the 15% solution showed evidence of the ψ form. In the Peg 8 and Peg 3 media even the 10% w/v samples had features of the condensed form present. In Figure 2 dotted lines are indicated to delineate regions of concentration for which the samples show CD spectra with ψ form features.

A number of authors have interpreted the effects of poly(ethylene glycols) on equilibria and kinetics of macromolecular reactions in terms of excluded-volume effects.[5,9] For example, Louie and Serwer have studied the cyclization or bimolecular joining of the "sticky" ends of phage λ-DNA in the presence of poly(ethylene glycols) of different molecular weights and find that the effects of the glycols on the processes are accounted for by the excluded volumes of the polymers.[6] Other reactions involving nucleic acids for which poly(ethylene glycol) crowding effects have been studied are reviewed in Zimmerman.[5] While the effects of poly(ethylene glycols) can be complex, the excluded-volume contributions are delineated by assuming that in equilibrium reactions if the volume of the initial state is smaller than that of the final state, then the initial state will be stabilized in the presence of molecules which occupy significant volume in solution. In the case of the melting of poly(dT)−poly(dA)−poly(dT), triplex stabilization by the polymeric glycols is clearly evident from the increases in T_m with increased concentration of glycol. The unraveling of triplex or duplex DNA should result in a volume increase due to the formation of random coils, which in the presence of poly(ethylene glycols) will exclude significant volume.[8] Excluded-volume effects can also account for the formation of the condensed ψ phase at higher polymer concentrations. As the solution becomes more crowded, the triplex (or duplex) is forced to occupy less volume, and thus condensation would be favored. The ψ phase of duplex DNA has been analyzed as a kind of liquid crystalline polymorphic aggregate that has long-range order along the helical axis.[10] It is thus reasonable that solution crowding helps to stabilize these polymorphic forms.

The observed magnitude of the shifts in T_m for the triplex relative to the duplex deserves further comment. The increases in duplex melting temperature for higher molecular weight Peg's are less than 2 deg for about 10% w/v solutions. On the other hand, the triplex melting transitions are almost 10 deg higher in the same media. (See Figure 2). Simple geometric models for melting of duplex DNA would involve a cylindrical helix melting to two random-coiled, single strands, while triplex melting would be from one cylindrical helix to a thinner cylinder plus a single random coil.[8] These simple models would predict that the duplex ought to have a larger increase in excluded volume than the triplex, since two random coils are produced rather than just one. The experimental results presented here show just the opposite, the triplex being more highly stabilized than the duplex. Thus, although excluded-volume increases can account for the increased stability of duplex or triplex DNA in poly(ethylene glycol) media, they do not account for the differences between duplex and triplex melting behavior.

One might argue that changes in water activity are responsible for the observed differences in melting behavior of duplex and triplex DNA. The melting transitions in ethylene glycol, which is frequently used to decrease solution water activity,[5] show that duplex melting is more affected by lower water activity than the triplex. Increasing ethylene glycol concentrations cause an almost 7 deg decrease in melting temperature for the duplex, while the triplex transition is lowered only 2.5 deg. In duplex DNA bound water has been identified, particularly in the minor groove of the helix.[11] If this bound water were released on melting, it would follow that decreased water activity would destabilize the helix and lower the transition temperature of duplex melting, as is observed. The fact that the triplex is affected less by decreased water activity suggests that fewer bound waters reside in the triplex, perhaps as a result of the presence of the third strand in the major groove of the duplex. Thus, it is possible that the observed effect of poly(ethylene glycols) is a composite of crowding and water activity factors operating together. Further work is required to sort out the effects of these agents on DNA helical structures.

Acknowledgment. Supported by Grant CA35635 from the National Cancer Institute (J.B.C.).

Supporting Information Available: Plots of ΔT_m as a function of % Peg 3400 and % Peg 04 (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA952427P

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