Binding Studies of a Triple-Helical Peptide Model of Macrophage Scavenger Receptor to Tetraplex Nucleic Acids†

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ABSTRACT: The macrophage scavenger receptor (MSR), involved in the uptake of oxidized LDL, binds a variety of polyanionic ligands, and in particular shows selectivity for tetraplex forms of nucleic acids. The ligand binding region has been shown to lie in the triple-helical collagen-like domain of MSR. A model peptide−nucleic acid system is presented here to clarify how the triple-helical motif of MSR recognizes and binds tetraplex nucleic acids. The triple-helical peptide MSR-1, with the sequence $(POG)_3PKGQKGEKG(POG)_4$, contains a nine amino acid basic sequence implicated in MSR ligand binding, flanked by Pro-Hyp-Gly triplets to provide stability. The ability of this triple-helical MSR-1 peptide to bind to and perturb the conformation of nucleic acids in tetraplex, duplex, and single-stranded states was assessed by monitoring changes in the nucleic acid circular dichroism spectrum in the 240−300 nm region. Our results show that the triple-helical MSR-1 peptide binds to tetraplex poly(I) in a stoichiometric manner and is capable of reproducing the discrimination exhibited by the native MSR molecule for tetraplex over double-stranded or single-stranded nucleic acid states. The triple-helical reference peptide $(POG)_{10}$ does not bind to tetraplex poly(I), suggesting that binding requires the highly basic 9-mer sequence from MSR that is included in MSR-1. The MSR-1 peptide did not perturb the CD spectra of a series of other tetraplex nucleic acids, indicating that it does not model the broader specificity that MSR shows under physiological conditions. Models of possible interactions between a triple-helical peptide and a tetraplex polynucleotide are proposed on the basis of the stoichiometry observed for the complex between triple-helical MSR-1 and tetraplex poly(I).

Macrophage scavenger receptors (MSR)[1] are trimeric membrane glycoproteins which mediate the uptake of oxidized LDL (Brown & Goldstein, 1983; Kodama *et al.*, 1990; Krieger, 1992; Krieger *et al.*, 1993). MSR-mediated endocytosis of oxidized LDL by macrophages has been implicated in the development of plaques in the intima of arteries in familial hypercholesterolemia and may be of general importance in the etiology of atherosclerosis (Goldstein *et al.*, 1979; Brown & Goldstein, 1983). Although MSR was first identified as participating in the uptake of oxidized LDL, it subsequently has been found to bind to a variety of polyanionic ligands (Brown & Goldstein, 1983; Resnick *et al.*, 1993; Pearson *et al.*, 1993), including certain modified proteins, phospholipids, polysaccharides, and some nucleic acids. Despite such a wide spectrum of ligands, MSR shows significant discrimination in binding. For instance, MSR will not bind to LDL or bovine serum albumin, but will bind oxidized LDL, acetylated LDL, and maleylated bovine serum albumin, presumably as a result of their increased negative charge. Dextran sulfate serves as a ligand, while chondroitin sulfate does not. MSR will mediate the uptake of poly(I) and poly(G), but not poly(C), poly(U), or poly(A) (Brown & Goldstein, 1983). It is believed that the nucleic acid selectivity exhibited by MSR is conformational in nature, particularly being related to the state of nucleic acid association since only tetraplexes are recognized (Pearson *et al.*, 1993). Clearly, understanding the basis for the ligand selectivities exhibited by MSR is important for comprehending its biological function, as well as posing a challenge for our understanding of ligand−receptor interactions. The results reported here contribute toward this understanding, while presenting a general model for interactions/recognition between a triple-helical peptide and a tetraplex nucleic acid.

MSR molecules are multidomain proteins which contain a transmembrane segment, a coiled-coil α-helical region, and a collagen-like triple-helical domain (Kodama *et al.*, 1990). Deletion and substitution studies have been carried out on MSR to identify the roles of different domains. The coiled-coil α-helical region has been implicated in the trimerization of the three chains and in endosomal ligand−receptor dissociation (Doi *et al.*, 1993, 1994; Acton *et al.*, 1993). The

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[1] Abbreviations: MSR, macrophage scavenger receptor; LDL, low-density lipoprotein; CD, circular dichroism; standard three-letter and one-letter abbreviations are used for the common amino acids, with hydroxyproline denoted by Hyp (three-letter code) and O (one-letter code).

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region involved in ligand binding has been identified as the collagen-like domain (Doi *et al.*, 1993; Acton *et al.*, 1993), which in human MSR consists of a 69 residue sequence with Gly as every third residue, $(Gly\text{-}X\text{-}Y)_{23}$, and a high content (20%) of imino acids (Matsumoto *et al*., 1990). These features meet the strict sequence constraints for a triple helix (Rich & Crick, 1961; Fraser & MacRae, 1973). The collagen-like region of MSR is highly charged (22% charged residues), and contains twice as many basic residues as acidic residues. Lysine residues in the Y positions of Gly-X-Y triplets near the C-terminus of the MSR triple helix have been implicated in ligand binding (Doi *et al*., 1993). Electrostatic interactions likely form the basis for binding of the positively charged triple helix to negatively charged ligands, with additional interactions (e.g., global conformational fits or sequence-dependent functional groups) being required to explain the specificity exhibited for different polyanion ligands (Brown & Goldstein, 1983; Resnick *et al.*, 1993; Pearson *et al*., 1993).

In this work, we design and study a model peptide−nucleic acid system to probe the basis for recognition and binding of polyanionic ligands by the triple-helical domain of MSR. A recently synthesized triple-helical peptide (Anachi *et al*., 1995), designated MSR-1, contains a nine amino acid sequence found near the C-terminus of the human MSR triple-helix region that has been shown to be important for ligand binding (Doi *et al.*, 1993). This MSR-1 peptide adopts a stable triple-helical conformation at neutral pH (Anachi *et al*., 1995). We spectrophotometrically assayed the ability of this MSR-1 peptide to bind to nucleic acids of different sequences in single-stranded, double-stranded (duplex), or four-stranded (tetraplex) forms. Because of their known ability to form tetraplex structures under specified temperature and salt conditions (Arnott *et al*., 1974; Pilch *et al*., 1995; Zimmerman *et al*., 1975), poly(I), poly(G), and oligonucleotides rich in inosine or guanosine were used. Nucleic acids known to be in single-stranded or double-stranded states were also examined. The results obtained indicate that triple-helical MSR-1 peptide discriminates between nucleic acids in a manner that depends on the amino acid sequence of the receptor peptide, and the composition/conformation of the nucleic acid ligand. Specifically, we find an interaction between the triple-helical MSR-1 peptide and the tetraplex poly(I) nucleic acid. We propose a model that is consistent with this interaction and its measured stoichiometry.

MATERIALS AND METHODS

The peptide $(POG)_3PKGQKGEKG(POG)_4$, referred to as MSR-1 peptide, was synthesized by solid-state peptide synthesis on an Applied Biosystems 430A peptide synthesizer using a standard *t*-Boc protection strategy on *t*-Boc-L-Gly-PAM resin, and was purified on HPLC, as previously described (Anachi *et al*., 1995). Poly(I) (average length 285 bases) and all other polynucleotides were purchased from Pharmacia Biotech Inc. (Piscataway, NJ). The deoxyoligonucleotide $d[T(G)_3TT(G)_3T]$ was synthesized on a solid support using the automated H-phosphonate method (Gaffney *et al.*, 1992) and purified by HPLC (Pharmacia LKB), followed by ion-exchange chromatography (AG 50W-X2 resin from Bio-Rad, Richmond, CA). The oligoribonucleotide r(UGGGU) was synthesized manually, on a solid support using phosphoramidite method and 2′-*O*-tetrahydropyranyl protection groups (Mielewczyk and Breslauer, personal communication; Kierzek *et al*., 1986). Purification and ion exchange were done as for oligodeoxynucleotides. Salts used for buffer preparation were obtained from Aldrich (Milwaukee, WI).

The buffer solution typically used for tetraplex experiments contained 0.1 mM K-EDTA, 10 mM potassium phosphate, and 100 mM KCl at pH 7.0. The low-salt buffer used for single-stranded poly(I) experiments contained 0.1 mM Na-EDTA and 5 mM sodium phosphate at pH 7.0.

UV absorption spectra were measured on an AVIV 14DS spectrophotometer (AVIV, Lakewood, NJ), and CD spectra were measured on an AVIV 60DS spectropolarimeter equipped with a Peltier temperature controller (AVIV).

CD titration experiments were used to assay for the binding of peptide to nucleic acids. The 200−240 nm region of the CD spectrum contains peaks of the peptide as well as from nucleic acids, but the 240−300 nm region contains exclusively peaks from nucleic acids. We monitored the maxima and minima of peaks in the 240−300 nm region as a function of increasing concentration of the peptide, while keeping the concentration of nucleic acid unchanged. Changes in the amplitudes of these peaks suggest conformational changes in the nucleic acid caused by the presence of the peptide, and were used as an indication of binding.

In a typical CD titration experiment, we used two solutions (A and B), each containing the same concentration of the nucleic acid dissolved in 10 mM potassium phosphate buffer with 100 mM KCl. The concentration of nucleic acid, calculated per base, was 0.21 mM, which corresponds to 0.052 mM per tetrad in the case of a tetraplex-forming nucleic acid. This gives an optimal absorption of 1.0−1.2 in a 10 mm UV cuvette, ensuring a strong signal for CD measurements. Solution B contained the peptide (concentration: 0.1 mg/mL; 12 μM triple helix), in addition to the nucleic acid. Both solutions were left overnight at 5 °C to equilibrate. The following day, the CD spectrum of solution A was recorded at 10 °C, and then one positive peak and one negative peak in the 240−300 nm region was monitored during the course of titration with solution B. The difference between the positive peak and the negative peak were monitored during the titration to minimize data manipulation. The CD spectrum was taken every 30 min after addition of a 50−100 μL aliquot of solution B to solution A. Three repeat scans and 5 s of averaging time were used to get the best quality of the data. The spectra were further smoothed using the AVIV algorithm before data analysis. The use of the difference between the positive and negative peaks and the presence of the same concentration of nucleic acid in both A and B solutions are features which enhance the ability to monitor small changes in the CD spectra.

RESULTS

Conformation of $(Pro\text{-}Hyp\text{-}Gly)_{10}$ and MSR-1 Peptide. Peptides with Gly as every third residue and a high content of the imino acids Pro and Hyp [e.g., $(Pro\text{-}Hyp\text{-}Gly)_{10}$] adopt a triple-helical conformation (Sakikabara *et al*., 1973; Engel *et al.*, 1977; Long *et al*., 1993; Bella *et al*., 1994). The peptide MSR-1 was designed to replace the central three Pro-Hyp-Gly triplets of $(Pro\text{-}Hyp\text{-}Gly)_{10}$ by residues 333−341 of the human macrophage scavenger receptor, yielding a final sequence of $(POG)_3PKGQKGEKG(POG)_4$ (Anachi *et al*.,

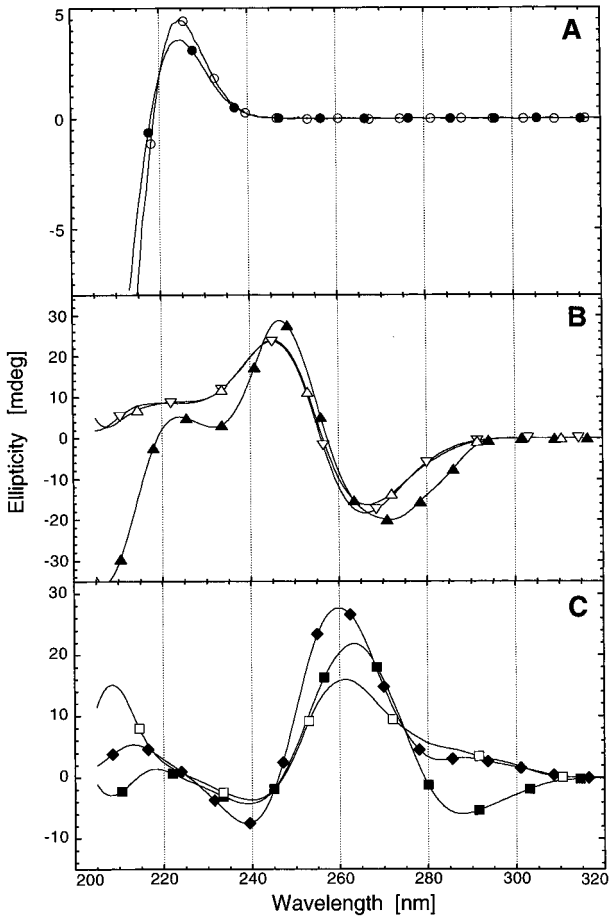

FIGURE 1: Circular dichroism spectra of the following: (A) peptide (Pro-Hyp-Gly)$_{10}$ (○) and MSR-1 (●) (concentration, 7.5 μM single-strand peptide); (B) inosine-containing nucleic acid tetraplexes, poly(I) (▲) (105 μM base concentration), poly[d(I)] (▽) (210 μM base), d[T(I)$_{12}$T] (△) (280 μM base), (C) guanosine-containing nucleic acid tetraplexes, poly(G) (♦) (150 μM base), r(UGGGU) (■) (75 μM base), and d[T(G)$_3$TT(G)$_3$T] (□) (75 μM base). All spectra were recorded at 10 °C in 10 mM phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 100 mM KCl. Concentrations of nucleic acids were varied as noted to allow easy visual comparison of the diagnostic shapes of the spectra.

1995). This MSR sequence contains lysyl residues at three consecutive Y positions, a feature which is very unusual in triple-helix proteins. Significantly, these periodic basic residues are implicated in MSR ligand binding (Doi *et al*., 1993). The circular dichroism (CD) spectra of (POG)$_{10}$ and the MSR-1 peptide at 10 °C (Figure 1A) both show a maximum near 225 nm, which is characteristic of a triple-helix structure, and this peak is decreased or obliterated at high temperatures (Anachi *et al*., 1995). Note that the peptide spectra shown in panel A of Figure 1 exhibit no circular dichroism features at wavelengths higher than 240 nm. When equilibrium CD spectra at pH 7.0 are recorded at increasing temperatures (Figure 2A), MSR-1 shows a sharp thermal transition, consistent with a trimer to monomer transition, and the melting temperature is 29.7 °C (panel C of Figure 2). The MSR-1 peptide is significantly less stable than (POG)$_{10}$, which has a melting temperature of 60 °C (Sakikabara *et al*., 1973; Engel *et al*., 1977). The thermal stability of (POG)$_{10}$ shows a small dependence on pH ($\Delta T_m \approx 6$ °C) (Venugopal *et al*., 1994), while the thermal stability of MSR-1 peptide is highly dependent on pH. Specifically, at low pH, the MSR-1 triple-helical peptide is thermally destabilized by more than 30 °C relative to pH 7.0 (Anachi *et al*., 1995). This observation is consistent with a strong contribution to triple-helix stability from electrostatic interactions, a reasonable expectation considering the highly charged nature of the interacting species.

Tetraplex-Forming Nucleic Acids. Polynucleotides and oligonucleotides can adopt many conformations, depending upon base sequence, pH, salt conditions, and temperature. Nucleic acids may be found in single-stranded forms, double-stranded forms (e.g., A, B, and Z), triple-helix forms, and tetraplex forms (Williamson, 1993; Cheng & Pettitt, 1992; Plum *et al*., 1995). In this work, we primarily used nucleic acids that favor tetraplexes for our MSR-1 binding studies since previous studies suggested that MSR binds specifically to nucleic acids in a tetraplex conformation (Pearson *et al*., 1994). Such nucleic acids include the polynucleotides poly(I) and poly(G) and oligonucleotides rich in G and I bases (Jin *et al*., 1992; Arnott *et al*., 1974; Zimmerman *et al*., 1975). Polynucleotides that form double helices or favor single strands also were used to probe the stringency of the tetraplex requirement for MSR-1 binding.

Initial studies were carried out on poly(I). At low temperature, the CD spectrum of poly(I) shows a minimum at 272 nm, a maximum at 247 nm, a smaller maximum at 224 nm, and a minimum near 207 nm (Figure 1B). The features of this spectrum previously have been reported to be characteristic of the tetrad form of poly(I) (Howard & Miles, 1982). Monitoring of the peak at 275 nm as a function of temperature indicated a complex set of four transitions, with the characteristic tetraplex features seen for temperatures 5, 15, and 25 °C but not at higher temperatures (Figure 2D).

CD spectra similar to that for tetraplex poly(I) were found at low temperature for poly[d(I)] and the oligonucleotide d[T(I)$_{12}$T] (Figure 1B). The CD spectra of the polynucleotide poly(G), the oligoribonucleotide r(UGGGU), and the oligodeoxynucleotide d[T(G)$_3$TT(G)$_3$T], as shown in Figure 1C, are also consistent with tetraplex forms (G-DNA) (Jin *et al*., 1992; Pearson *et al*., 1993; Mielewczyk and Breslauer, personal communication). With increasing temperature, the CD extrema of all of these nucleotides except for d[T(I)$_{12}$T] exhibit transitions characteristic of tetraplex to monomer transformations (data not shown). In contrast, the CD spectrum of the oligonucleotide d[T(I)$_{12}$T] shows a gradual diminishing of peak intensity with increasing temperature.

Binding of MSR-1 to Tetraplex Poly[r(I)]. The CD spectrum of tetraplex poly(I) has a strong minimum (272 nm) and a strong maximum (247 nm) in the wavelength region where the MSR-1 peptide shows no CD signal (Figure 1). A circular dichroism assay was designed to measure the effect of adding MSR-1 peptide on the poly(I) tetraplex CD signals in this 240−300 nm region. Observation of changes occurring in a stoichiometric way in the CD peaks of poly(I) in the 240−300 nm region upon addition of the peptide would be a strong indicator that the MSR-1 peptide is binding to poly(I) and perturbing the poly(I) conformation.

Titration of a tetraplex poly(I) solution with the MSR-1 peptide results in changes in the intensity of the poly(I) CD peaks at 272 and 247 nm. As shown in Figure 3, a plot of the change in the ellipticity of these two poly(I) peaks as a function of peptide concentration shows a strong linear dependence up to 3 μM, followed by a plateau at higher peptide concentrations. This behavior is consistent with the triple-helical MSR-1 peptide binding to tetraplex poly(I) such

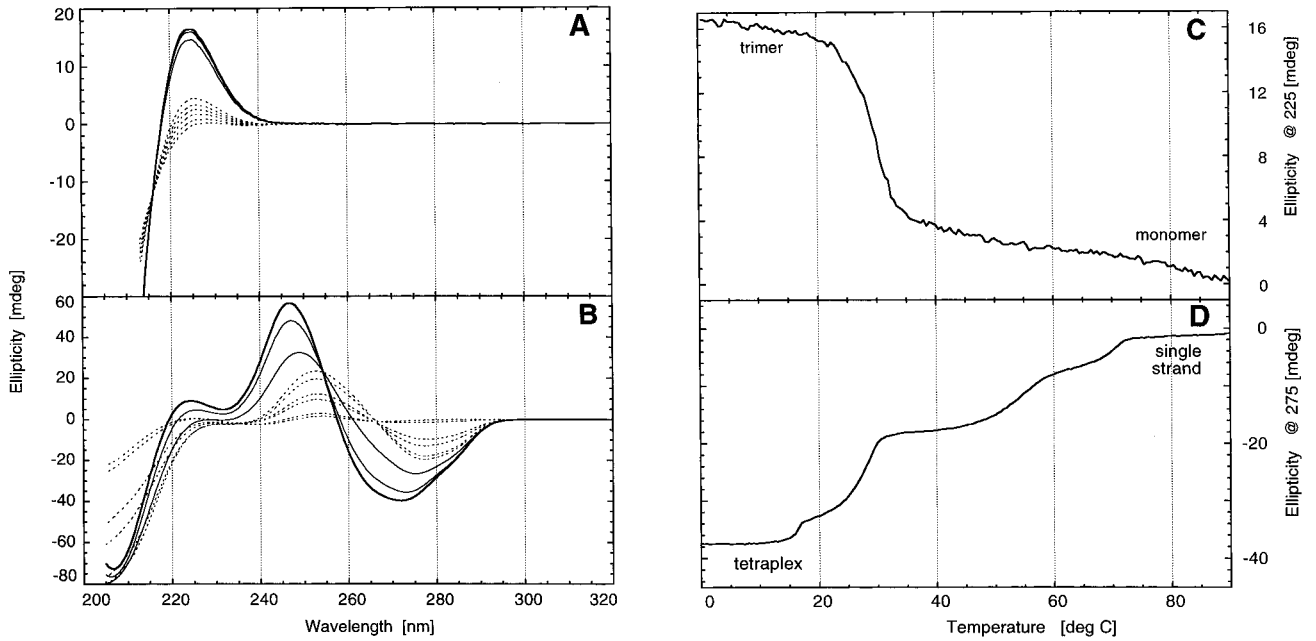

FIGURE 2: Circular dichroism spectra of (A) MSR-1 peptide and (B) poly(I) at temperatures ranging from 5 to 85 °C. In (A), the spectral curves of the MSR-1 peptide (concentration: 36 μM single-strand peptide) are shown in order of increasing temperature, with a 10 °C increase between adjacent curves; the 5 °C spectrum has the highest magnitude peak at 223 nm, while the 85 °C spectrum has the lowest maximum at 223 nm. The spectra of MSR-1 peptide which are in a triple-helical form at 5, 15, and 25 °C are solid lines, while the spectra of MSR-1 peptide in a monomer form at 35, 45, 55, 65, 75, and 85 °C are dashed lines. In (B), the spectra of poly(I) (concentration: 210 μM base) are shown in order of increasing temperature, with a 10 °C increase between adjacent curves; the 5 °C spectrum has the largest magnitudes at the maximum at 247 nm and minimum at 272 nm, while the 85 °C spectrum has the lowest magnitudes at the extrema. The spectra of poly(I) which show characteristic tetraplex features are indicated by solid lines (5, 15, and 25 °C), while the others are indicated by dashed lines (35, 45, 55, 65, 75, and 85 °C). The thermal transitions obtained from monitoring one wavelength as a function of temperature are shown for (C) MSR-1 at 225 nm and (D) poly(I) at 275 nm. The spectra were recorded in 10 mM phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 100 mM KCl. Note that the CD melting profile of poly(I) reveals that the transition from a tetraplex form to a single stranded one is not a simple process, but consists of at least four consecutive events and involves the formation of intermediate species with unknown conformations.

that this binding is stoichiometric and perturbs the conformation of the tetraplex. We cannot eliminate the possibility that some of the signal above 240 nm results from an induced CD signal from the MSR-1 peptide. Nevertheless, in either case, the CD changes are consistent with some form of stoichiometric binding.

It would be desirable to investigate if increased salt concentration could reverse the titration observed in Figure 3, to evaluate the electrostatic contribution to this interaction. Unfortunately, the tetraplex form of poly(I) is only stable in solution at 50, 75, and 100 mM KCl, and precipitation occurs at higher salt concentrations (data not shown).

Complex formation between the MSR-1 peptide and poly(I) might be expected to displace the cooperative thermal transition of each component to higher temperatures. The thermal profile of poly(I) was monitored at 275 nm, both alone (Figure 2D) and in the presence of MSR-1 peptide (data not shown). The melting profile of poly(I) alone shows a complicated equilibrium of different forms, with four transitions (Figure 2D), but only one of these transitions (midpoint near 15 °C) occurs at a temperature where the MSR-1 peptide is fully triple-helical (<22 °C). No major shifts in the poly(I) thermal transitions were seen as a result of complex formation. In particular, the transition near 15 °C was broadened but remained at the same midpoint. The absence of a T_m shift could reflect a lack of binding to the initial state, similar binding to the ensemble of initial and final states, or binding with a near-zero enthalpy change, thereby causing the equilibrium to be unperturbed by temperature. Interestingly, the similarity of the poly(I) spectra at 5, 15, and 25 °C (solid lines in Figure 2B) suggests that the 15 °C transition may represent a transition between two different tetraplex forms, both of which could bind to the MSR-1 triple-helical peptide, thereby resulting in the near-zero T_m shift. It is difficult to monitor changes in conformation or stability of the MSR-1 peptide as a result of binding to tetraplex poly(I), since both the nucleic acid as well as the peptide have a CD signal in the 200−230 nm region.

Similar titration studies of poly(I) with MSR-1 peptide were carried out under conditions where poly(I) was present in a double-stranded state, as part of the poly(I)·poly(C) duplex (open circles in Figure 3). Note that unlike the results with tetraplex poly(I), no change is seen in the nucleic acid CD spectra as a result of the peptide addition. There was also no change in the nucleic acid CD signal and therefore no indication of MSR-1 binding when poly(I) was added under low-salt conditions where it assumes a single-stranded state (filled squares in Figure 3). In the aggregate, these results suggest a conformational specificity for MSR-1 binding in which the tetraplex state is preferred.

Lack of Binding of Tetraplex Poly(I) to (Pro-Hyp-Gly)$_{10}$. To test for a specific amino acid sequence requirement in the triple-helical peptide, or whether the triple-helix conformation itself is sufficient, the spectroscopic binding assay described above was carried out using the triple-helical peptide (Pro-Hyp-Gly)$_{10}$. In contrast to the results seen with MSR-1, no change was observed in the CD spectrum of tetraplex poly(I) upon titration with (POG)$_{10}$ (Figure 4). This suggests (POG)$_{10}$ does not bind to the poly(I) tetraplex.

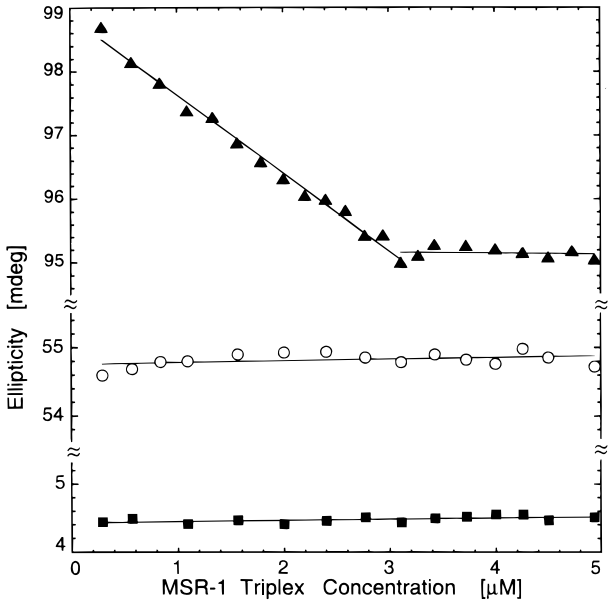

FIGURE 3: Titration of different forms of nucleic acids containing poly(I) with the triple-helical MSR-1 peptide. The ellipticity difference between the maximum near 240 nm and the minimum near 270 nm is plotted as a function of MSR-1 trimer peptide concentration (μM) for the tetraplex poly(I) (▲), the double-stranded poly(I)·poly(C) (○), and the single-stranded poly(I) in 5 mM sodium phosphate (■). All titrations were carried out at 10 °C, with a nucleotide concentration of 210 μM. Note that the scale is broken to accommodate the different values for the ellipticity differences of the three nucleic acid forms.

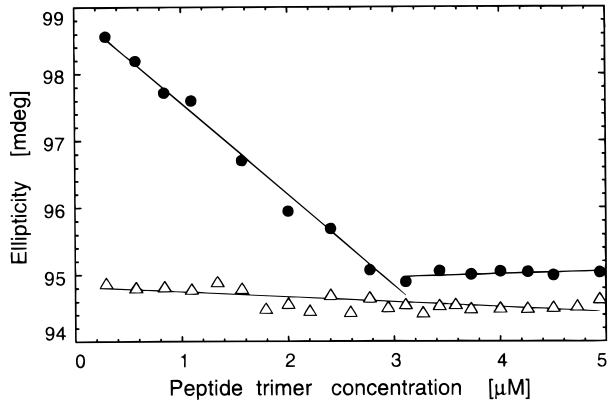

FIGURE 4: Titration of tetraplex poly(I) with peptide (Pro-Hyp-Gly)$_{10}$ (△), plotting the ellipticity difference between the 245 nm maximum and the 272 nm minimum as a function of peptide trimer concentration. A competition assay is also shown (●), where poly-(I) is incubated with 5 μM trimer (Pro-Hyp-Gly)$_{10}$ overnight, followed by titration with MSR-1 trimer peptide. Here the ellipticity difference is plotted as a function of MSR-1 trimer concentration. The MSR-1 titration curve observed when (Pro-Hyp-Gly)$_{10}$ is included is virtually identical to that obtained in the absence of (Pro-Hyp-Gly)$_{10}$ (see Figure 3).

Alternatively, binding may occur but without perturbing the CD spectrum, an unlikely possibility that nevertheless *a priori* cannot be eliminated.

To investigate the possibility that (POG)$_{10}$ is binding to poly(I) without causing a detectable change in the poly(I) CD spectrum, a competition binding experiment was carried out. MSR-1 peptide was added in increasing amounts to a solution containing both (POG)$_{10}$ and tetraplex poly(I), and the poly(I) CD peaks (272 and 247 nm) were monitored. The resulting titration curve was indistinguishable from that obtained by adding MSR-1 to tetraplex poly(I) in the absence of (POG)$_{10}$ (Figure 4). This result suggests that tetraplex poly(I) does not bind (POG)$_{10}$, and that there is a specific amino acid sequence requirement in the triple-helical peptide for binding to occur.

Table 1: Classification of the Forms of Nucleic Acid Structures Investigated in Binding Studies with the MSR-1 Triple-Helical Peptide

tetraplex	duplex	single stranded
poly(I)	poly(I)·poly(C)	poly(I), low salt
poly[d(I)]	poly(AU)·poly(AU)	poly[d(A)]
poly(G)	poly[d(AT)]·poly[d(AT)]	poly(A)
d[T(I)$_{12}$T]	poly(A)·poly(U)	
d[T(G)$_3$TT(G)$_3$T]	poly[d(A)]·poly[d(T)]	
r(UGGGU)		

Lack of Perturbation of Other Tetraplex Nucleic Acids by the MSR-1 Peptide. MSR-1 peptide binding to a series of other nucleic acids which adopt a tetraplex conformation at low temperature also was investigated using our CD assay (Table 1). Specifically, CD spectra of poly[d(I)], poly(G), d[T(I)$_{12}$T], d[T(G)$_3$TT(G)$_3$T], and r(UGGGU) were monitored during addition of increasing amounts of triple-helical MSR-1, with no changes being detected in the CD spectra of any of these tetraplexes (data not shown). These results are consistent with the nucleic acid tetraplex state alone not being sufficient for MSR-1 peptide binding, while suggesting that the poly(I) tetraplex possesses features of static and/or inducible structure that makes it uniquely susceptible to MSR-1 binding. As before, our CD assay does not eliminate the possibility of some MSR-1 binding to these other tetraplexes which does not result in a perturbation of the CD spectra.

Lack of Perturbation of CD Spectra of Double-Stranded and Single-Stranded Nucleic Acids by MSR-1 Peptide. To examine the stringency of the requirement for MSR-1 binding, we studied a series of nucleic acids in double-stranded states [poly(AU)·poly(AU), poly [d(AT)]·poly-[d(AT)], poly(A)·poly(U), and poly [d(A)]·poly[d(T)] and in single-stranded states [poly[d(A)] and poly(A)] (Table 1). None of these double-stranded or single-stranded nucleic acids showed any change in the CD spectra upon titration with the triple-helical MSR-1 peptide (data not shown).

DISCUSSION

Formation of a complex between a triple-helical peptide and a tetraplex nucleic acid offers an opportunity to examine the interactions of two multistrand helical conformations which have important physiological roles. Triple-helix domains in proteins bind to a variety of molecules as part of their biological function (Brodsky & Shah, 1995; Hoppe & Reid, 1994; Kadler, 1994). These include the binding of the collagen triple helix to integrins, collagenase, fibronectin, and heparin, as well receptor binding by the collagen-like domain of C1q and ligand binding by the collagen-like domain of MSR. Tetraplex nucleic acid structures are found in telomeres (Klobutcher *et al.*, 1981; Zakian *et al.*, 1989; Henderson & Blackburn, 1989), which are critical to the stability and integrity of chromosomes (Blackburn, 1991). Guanine-rich sequences, which are characteristic of tetraplex formation, are also present in gene regulatory regions (Nickol & Felsenfeld, 1983; Evans *et al.*, 1984; Kilpatrick *et al.*, 1986; Murchie & Lilley, 1992). Interactions of proteins with tetraplex nucleic acids are suggested by the ability of some

basic proteins, as well as polylysine, to promote the formation of tetraplex structure of telomeres (Fang & Cech, 1993). Recognition and binding of a relatively uniform triple-helical protein to a tetraplex nucleic acid could involve both conformational and sequence features, and the participation of helical structures could define specificity of a less discriminating nature than seen for globular protein interactions.

Our results show that a small model triple-helical peptide containing only nine residues from MSR binds to tetraplex poly(I) and is capable of reproducing the discrimination exhibited by the native MSR molecule between the tetraplex form relative to both single-stranded and double-stranded forms. The 69 residues in the collagen-like ligand binding region of human MSR adopt a triple-helical conformation and contain 10 positively charged residues, mostly in the Y positions of the Gly-X-Y repeating structure. The triple-helical 30-mer peptide, MSR-1, with three Lys residues in the Y positions of its central nine residues, can bind and have the same conformational discrimination as MSR. The essential nature of the Lys residues and the GPKGQKGEK sequence for binding to tetraplex poly(I) is shown by the lack of binding of the stable triple-helical peptide $(Pro\text{-}Hyp\text{-}Gly)_{10}$. This indicates that the triple-helix conformation of the peptide is not sufficient in itself to result in binding, with specific positively charged Lys residues ($pK \sim 10.5$) being required. The collagen-like region of C1q, which includes a positively charged region, binds nucleic acids with a specificity similar to that seen for MSR (Acton *et al*., 1993).

Although we find that the MSR-1 triple-helical peptide reproduces the discrimination between tetraplex and other nucleic acid conformations seen for MSR in the case of poly(I), the peptide MSR-1 did not model the broader nucleic acid sequence specificity seen for binding by MSR. Poly(G) and oligonucleotides containing inosine (I) and guanosine (G) have been shown to be taken up by the cell through endocytosis via MSR, and direct binding of MSR to tetraplex forms of poly(I), poly(G), poly(riboxanthinylic acid), and oligomers $[d(T_4G_4)_4$, $d(G_4T_4)_5$, and $dG_n]$ has been shown (Pearson *et al.*, 1993). Yet poly(G), a variety of tetraplex oligonucleotides containing I and G, and even poly[d(I)] did not show any perturbation of their CD spectrum with increasing addition of MSR-1 peptide. It is possible that MSR-1 does not interact with these other tetraplex nucleic acids because of the small number of MSR residues included in this peptide, and that a peptide containing a longer MSR sequence would more accurately reflect native binding specificity. Alternatively, since the interaction is monitored here by changes in the nucleic acid CD spectrum, MSR-1 could bind but not cause any detectable spectroscopic perturbation of the nucleic acid. Poly(G) is known to form a more stable tetraplex than poly(I), which could indicate a more rigid conformation. It is possible that the MSR-1 peptide binds to poly(G) without any significant perturbation of its conformation and without measurable CD changes.

It is possible to speculate about the nature of the interaction between a triple-helix peptide and a tetraplex polynucleotide, each having their own distinctive symmetry (Figure 5). The estimated binding stoichiometry of the complex, calculated from Figure 3 using molar ratios, is 16 inosine tetrads per 1 MSR-1 triple helix. Assuming a distance of 3.4 Å between inosine tetrads (Arnott *et al.*, 1974), this corresponds to a 56 Å fragment of poly(I) interacting on average with each

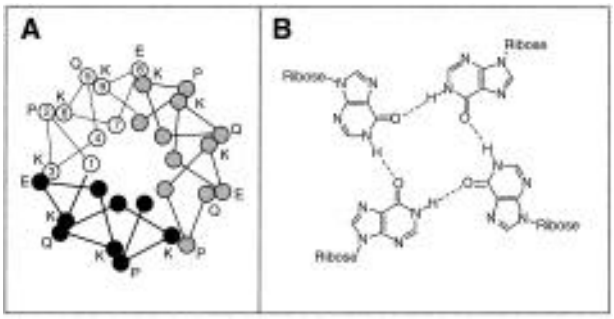

FIGURE 5: Schematic cross-section views of models of (A) MSR-1 triple-helical peptide and (B) inosine tetrad showing the hydrogen bonding. The model shown in panel A for the triple helix assumes a 10/3 screw (Fraser *et al.*, 1979), but a similar structure with a *7/2* symmetry is also possible (Bella *et al.*, 1994).

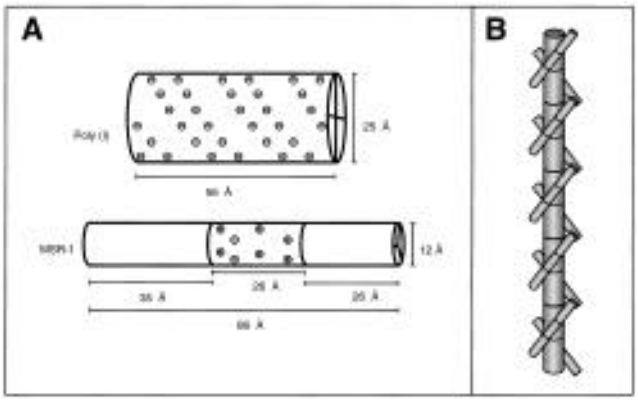

FIGURE 6: (A) Comparison of the dimensions of the interacting region of poly(I) and the size of a 30-mer peptide triple helix. The nine residue highly charged central region of MSR-1 is indicated. (B) Schematic representation of a possible way that poly(I) tetraplex (central, vertical cylinder) can interact with triple-helix peptide units (small, tilted cylinders).

86 Å long MSR-1 molecule (central 9 residues, 28 Å long). The relative sizes of the interacting regions on the triple helix and the tetraplex are shown schematically in Figure 6A. One possible model is to place the MSR-1 helix in the groove of the poly(I) tetraplex, with interactions between the phosphate groups and the lysyl groups (Figure 6B). Symmetry results in two equivalent grooves on opposite sides of the poly(I) tetraplex, but the calculated stoichiometry suggests that the rodlike triple-helical MSR-1 peptide molecules can only occupy one of the two grooves at a given time. The binding of MSR-1 peptide by poly(I) perturbs the tetraplex conformation sufficiently to be detected by changes in the CD signal. This perturbation of the tetraplex conformation caused by the binding of MSR-1 triple-helical peptide may result in a breaking of the symmetry, so that both sides of the tetraplex are no longer equivalent. Thus, the binding of one MSR-1 triple helix may have an allosteric effect on the other side of the poly(I) tetraplex such that it is no longer a significant binding site.

The broad specificity of MSR binding of polyanionic molecules suggests that the binding involves a general electrostatic attraction, but the discrimination between different polyanionic molecules indicates specific interactions as well. The observation that only tetraplex nucleic acids will bind to MSR shows the importance of stereospecific location of the negative charges. The MSR-1 peptide, with a nine residue charged lysine cluster implicated in ligand binding, mimics the conformational specificity but not the

sequence specificity of nucleic acid binding, and may serve as a model for understanding how a triple-helix motif can mediate recognition and binding. The observation of a specific interaction which is dependent both on the triple-helix amino acid sequence and the conformation and sequence of the nucleotide suggests an interesting system for studying protein−nucleic acid interactions, and for clarifying the nature of binding by these helical macromolecules.

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