Strandedness Discrimination in Peptide-Polynucleotide Complexes*

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Preferential binding to single- or double-stranded nucleic acids is important for the activity of many proteins that process RNA and DNA. We have investigated the mechanism of strandedness discrimination with peptides derived from the putative DNA-binding domain of the RecA protein, a bacterial recombinase that modulates its affinity for single-stranded DNA by means of ATP binding and hydrolysis. Contributions of electrostatic and non-electrostatic interactions to binding of these peptides with polynucleotides were evaluated by fluorescence spectroscopy as a function of salt concentration and peptide charge. Binding of these peptides to single- and double-stranded nucleic acids was dominated by non-electrostatic interactions. Small electrostatic contributions selectively enhanced peptide complexation with single-stranded nucleic acids. Similar results were observed in control experiments carried out with tripeptides containing charged and aromatic amino acid residues. It was possible to modify the strandedness preference of peptide-polynucleotide complexes by changing electrostatic contributions to the binding free energy. These observations suggest a mechanism whereby some proteins that interact with DNA or RNA might determine and regulate their relative affinity for single- and double-stranded nucleic acids.

Experimental Procedures
Preparation and Characterization of Peptides and Polynucleotides—Peptides wt, 659w, KWK, and AcKWK (Fig. 1b) were synthesized with an Applied Biosystems Model 430A apparatus by standard t-butoxycarbonyl chemistry and purified by reversed-phase HPLC on an Aquapore C8 column with a 0-70% acetonitrile gradient in 0.1% trifluoroacetic acid. Peptide purity was evaluated by HPLC, and molecular weights were confirmed by fast atom bombardment mass spectroscopy. Peptide stock concentrations were determined from Trp absorption at 280 nm in water at 25 °C after confirming that absorption of the peptides in water was the same as in 6 M guanidinium hydrochloride (17). Trp and polynucleotides were purchased from Sigma. Polynucleotides were exhaustively dialyzed against reaction buffer, and their concentrations were determined in 0.1 M Tris acetate, pH 7.4, at 25 °C using published extinction coefficients (18, 19). Solutions were prepared with filtered deionized water (Milli-Q, Millipore Corp.). Titrations were carried out in low salt buffer (10 mM Tris acetate, pH 7.4) or high salt buffer (1 M KCH3CO2, 10 mM Tris acetate, pH 7.4, for DNA, poly(A)-poly(U), and poly(dA); 0.2 M KCH3CO2, 10 mM Tris acetate, pH 7.4, for poly(A) and poly(dA)). Concentrations of peptides are expressed in units of moles of peptide molecule, whereas concentration units for polynucleotides are moles of nucleic acid residues.

Fluorescence Spectra and Titration—Fluorescence spectra were measured with an SLM 500 spectrophotometer (AMINCO). Samples were excited at 292 nm, and fluorescence was recorded as the integrated emission at 335-500 nm. Bandwidths for excitation and emission were 2 nm. UV absorbance and fluorescence of the peptide solutions varied linearly with peptide concentration, indicating the absence of association between peptide molecules in the concentration ranges used.

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During titration, aliquots of polynucleotide stock solution were added to peptide solutions. The samples were agitated and allowed to equilibrate in a thermostated sample holder at 25°C. In some experiments, titrations were carried out in low salt buffer, and the electrostatic contributions to the resulting complex were subsequently investigated by titrating with 2 M KCH₃CO₂ (or NaCH₃CO₂), 10 mM Trisacetate, pH 7.4. In other experiments, non-electrostatic binding was measured by titrating the peptide with polynucleotide in high salt buffer. Data are reported as fluorescence quenching, Q₀ₐ₅ is the initial fluorescence in the absence of polynucleotide and F is the fluorescence during titration. Fluorescence intensity was corrected for dilution, and inner filter effects were corrected by the relation \( F_{\text{corr}} = F_{\text{obs}} \cdot \exp(-2) \), where \( F_{\text{corr}} \) and \( F_{\text{obs}} \) are the corrected and observed fluorescence, respectively, and \( A_{\text{obs}} \) is the absorbance at the excitation wavelength (292 nm), which was calculated from the concentrations of polynucleotide and peptide with the following extinction coefficients: \( \varepsilon_{292} = 104 \text{ M}^{-1} \text{ cm}^{-1} \) (poly(A) and poly(dA)), \( \varepsilon_{292} = 160 \text{ M}^{-1} \text{ cm}^{-1} \) (poly(U)), \( \varepsilon_{292} = 850 \text{ M}^{-1} \text{ cm}^{-1} \) (DNA), \( \varepsilon_{292} = 183 \text{ M}^{-1} \text{ cm}^{-1} \) (peptides). Measurements during titration showed that the absorbance of polynucleotides in the reaction mixture obeyed Beer's law at 292 nm over the entire concentration range of the experiments. Experiments were carried in a 0.4 × 1.0-cm quartz cuvette with the 0.4-cm path oriented in the direction of the excitation beam in order to minimize absorbance corrections.

**FIG. 1.** Panel a, sequences of the L2 DNA-binding domains of homologous RecA-like proteins from phage T4 (Uvsx), Escherichia coli RecA (EcRecA), Saccharomyces cerevisiae Rad51 (ScRad51), and human Rad51 (HsRad51) proteins (8–10). Panel b, amino acid sequences of the peptides used in this study. Amino acids are presented by their single letter code.

**FIG. 2.** Fluorescence quenching of 24 μM peptide wtw (●) and peptide 659w (■) with poly(A) (a) and poly(U) (c) in 10 mM Tris acetate, pH 7.4, and fluorescence quenching at pH 7.4 of complexes formed in a and c upon addition of KCH₃CO₂ (b and d, respectively).

**RESULTS**

Electrostatic and Non-electrostatic Contributions to Stability of Peptide-Polynucleotide Complexes—Since titration of peptides with polynucleotides quenched the peptide fluorescence (Figs. 2–4), quenching can be used to measure the binding of peptide to polynucleotide. Both electrostatic and non-electrostatic interactions contributed to the binding of peptide 659w to single-stranded poly(A) and poly(U) at 25 °C, pH 7.4; this can be seen by the partial decrease in quenching of the peptide-polynucleotide solution with increasing KCH₃CO₂ (Fig. 2, squares). Complexes between peptide wtw and single-stranded nucleic acids were less sensitive to salt (Fig. 2, circles), indicating that they were stabilized to a lesser extent by electrostatic interactions. Non-electrostatic interactions stabilized the complexation of both wtw and 659w with double-stranded poly(A)-poly(U) and DNA in these reaction conditions without significant contributions from electrostatic binding (Fig. 3).
The incapacity of KCH$_3$CO$_2$ to disrupt the peptide complexes with polynucleotides primarily by electrostatic interactions (18, 19, 25–27). To investigate whether these differences with our observations were a consequence of particular peptide sequences or different reaction conditions, we compared the binding of KWK to poly(U) under our conditions at pH 7.4 with the same reaction at pH 5.8, which has been previously well studied (19).

The non-electrostatic nature of this binding is apparent from the incapacity of KCH$_3$CO$_2$ to disrupt the peptide complexes with poly(A)-poly(U) or DNA (Fig. 3) and from the similar titration curves observed in low and high salt buffers (data not shown). Furthermore, the fluorescence curves observed upon titration of 659w or wtw with double-stranded nucleic acids in low salt solutions were insensitive to the different charges of the nucleic acids. However, the fluorescence quenching of complexes formed in high salt solutions were insensitive to the different charges of the nucleic acids. Furthermore, the fluorescence curves observed upon titration of 659w or wtw with double-stranded nucleic acids in low salt concentrations only slightly diminished fluorescence quenching of the resulting complex. Hence, in contrast to peptide KWK, non-electrostatic interactions contributed selectively to the stability of complexes between peptide 659 and single-stranded polynucleotides; the calculated electrostatic binding constants were 2 orders of magnitude less than the non-electrostatic binding constants.

Effect of Peptide Sequence and Reaction Conditions on Non-electrostatic Interactions—It has been previously reported that short peptides of polylysine containing Trp residues bind to polynucleotides primarily by electrostatic interactions (18, 19, 25–27). To investigate whether these differences with our observations were a consequence of particular peptide sequences or different reaction conditions, we compared the binding of KWK to poly(U) under our conditions at pH 7.4 with the same reaction at pH 5.8, which has been previously well studied (19).
The nucleic acid-binding domains of proteins that preferentially recognize single-stranded nucleic acids are believed to be characterized by positively charged amino acid residues that interact electrostatically with the polynucleotide phosphate backbone and aromatic amino acids that can stabilize the complex through non-electrostatic interactions (2, 3, 6, 7). We have evaluated electrostatic and non-electrostatic contributions to the complexation of peptides with single- and double-stranded polynucleotides. The peptides studied were derived from the L2 DNA-binding domain of the RecA protein, a bacterial recombinase that cycles, in an ATP-dependent reaction, between conformations with high and low affinity for single-stranded DNA. These were compared with model tripeptides (KWK and AcKWK) that have the same charge as the L2 peptides (Fig. 1).

Two types of experiments showed that complexes of all peptides with double-stranded polynucleotides were stabilized by non-electrostatic interactions (Figs. 3 and 4 and Tables I and III). First, fluorescence quenching of peptides by poly(A)-poly(U) and DNA was insensitive to salt concentration. Second, altering the charge of the peptide did not affect binding to double-stranded nucleic acids; peptide charge was modified by either an amino acid substitution in the case of the L2 peptides or acetylation of the α-amino group of peptide KWK. Complexes of peptides with single-stranded polynucleotides were stabilized mainly by non-electrostatic interactions. Recent filter binding studies have also shown that peptides based on the amino acid sequence of the L2 loop of the RecA protein bound to single-stranded DNA at high salt concentrations and neutral pH (28) presumably by non-electrostatic interactions. However, we observed that the stability of single-stranded complexes was also partially sensitive to the charge of the peptide and to salt concentration (Table I). Hence, in our experiments, non-electrostatic interactions stabilized peptide complexes with both single- and double-stranded polynucleotides; electrostatic interactions, on the other hand, contributed selectively to the stability of single-stranded complexes.

Early fluorescence studies (26, 29) reported that peptide KWK bound to single- and double-stranded polynucleotides at low salt concentrations primarily by electrostatic interactions. The observed preference of KWK for single-stranded nucleotides (26, 29) likely reflects the larger electrostatic contributions to the stability of these complexes, compared with double-stranded polynucleotides, and is consistent with our results. However, in those experiments, inner filter effects were evaluated from fluorescence quenching of the reactants in solutions with high salt concentrations where complexes were assumed to entirely dissociate. Correcting for inner filter effects by this procedure could subtract potential non-electrostatic interactions from titrations at low salt concentrations, which may in part explain the reported absence of non-electrostatic binding.

Oligolysine peptides (K)ₙ-ε-N-DNP-K (n = 3–8) (24, 30) and KWKₙ (n = 1–8) (25) have been shown to bind to polynucleotides by an entirely electrostatic process. We also observed that non-electrostatic contributions did not significantly stabilize complexes between single-stranded poly(U) and KWK at pH 5.8 in low salt solution (Table II) as previously reported (19, 25). However, deprotonation or acetylation of the α-amino group of KWK or high salt concentrations increased the non-electrostatic contribution to this reaction (Fig. 4 and Tables I–III). These observations suggest that in low salt solutions at pH 5.8, KWK binds to polynucleotides through the positively charged α-amino group. Interactions between the α-amino group and the polynucleotide may explain why peptides with a total charge of z < +4 bind to single-stranded polynucleotides with different thermodynamics than more highly charged oligolysine molecules (25). Taken together, these results show that peptides with an uncharged α-amino extremity and a small net positive charge bind to polynucleotides in low salt solutions qualitatively differently than highly charged oligolysine molecules. Their large non-electrostatic interactions compared with those of polycyline oligopeptides suggest different peptide-nucleic acid contacts in the two cases.

One important consequence of these results is that electrostatic contributions to the binding free energy can modulate the strandedness preference of peptide-nucleic acid complexes. For
example, reducing the net positive charge of the peptide by an amino acid substitution (659w → wt) had no effect on peptide binding to double-stranded poly(A)-poly(U) at pH 7.4, but decreased binding to the component single-stranded polynucleotides poly(A) and poly(U) (Figs. 2 and 3 and Table I). Likewise, neutralization of the positive charge of the α-amino group (KWK → AcKWK) had little effect on binding to double-stranded DNA at pH 5.8, but decreased binding to single-stranded poly(U) more than with double-stranded poly(A)-poly(U) (Table III). Similarly, at pH 7.4, electrostatic interactions stabilized complexes of peptide KWK with poly(A) or poly(U) more than with double-stranded poly(A)-poly(U) (Fig. 4 and Table I).

These results suggest that, at least for certain peptide-polynucleotide complexes, increasing electrostatic binding can selectively enhance the affinity of the peptide for single-stranded nucleic acids. It should be noted that this observation cannot be explained by the relative charge density of single-stranded nucleic acids, which is lower than that of duplex molecules (27).

If these results can be extrapolated to protein-nucleic acid complexes, then protein conformational changes that modify the net charge of the binding domain could regulate strandedness preference of the protein. We note, for example, that negatively as well as positively charged amino acids are present in homologous DNA-binding domains of recombinases from phage to man (Fig. 1a) (8–10); negative charges in the DNA-binding domain might allow these proteins to modulate their affinity for single-stranded DNA by means of allosteric conformational changes that modify the relative electrostatic and non-electrostatic contributions to polynucleotide binding. Alteration of the relative affinity of the L2 loop of the RecA protein for single- and double-stranded DNA could have important consequences for the mechanism of action of the RecA-DNA nucleoprotein filament during homologous recombination.

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