Spermine-Induced Conformational Changes of a Synthetic Peptide

Mike Tabet,[†] Virender Labroo,[‡] Paul Sheppard,[‡] and Tomikazu Sasaki*,[†]

Contribution from the Department of Chemistry, University of Washington, Seattle, Washington 98195, and Zymogenetics, 4255 Roosevelt Way NE, Seattle, Washington 98105

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Abstract: A 14-residue peptide, SBP, was synthesized to study the interactions between polypeptides and biological polyamines, e.g. spermine and its derivatives. Four glutamic acid residues were placed in the sequence in such a way that binding of spermine to the peptide could induce the α-helical conformation. SBP was found to form a stable 1:1 complex with spermine in neutral aqueous buffer containing 30% trifluoroethanol at 4 °C. The α-helicity (19%) of SBP increased to 38% in the presence of spermine. The increase in α-helicity of SBP was strongly dependent on pH with a maximum around pH = 7, suggesting ionic interactions between SBP and spermine. Other structurally related polyamines including spermidine and putrescine were also examined to study the specificity of the complex formation. A number of similar sequence motifs that could interact with spermine were identified in native proteins registered in the protein data bank.

Introduction

The α-helical conformation is an essential structural motif for many biologically active peptides and proteins. Several small peptides[1,8] have been designed to adopt a stable α-helical conformation. Ion pairs and metal coordinations as well as covalent bonds were used to provide specific inter-residue interactions. In these systems, enforced interactions between side chains of (i) and ($i + 4$) or (i) and ($i + 7$) residues appear to initiate the formation of a local α-helix structure that propagates through the rest of the peptide chain.

Cooperative interactions of multiple residues at these specific positions would further stabilize the helical conformation. Under physiological pH, a biological polyamine, spermine, exists as a protonated form with four positively charged amino groups that are separated by three, four, and three carbons, respectively.[2] Distances between these positive charges in the extended conformation are 4.6, 6.0, and 4.6 Å and are comparable to the translation per turn (5.5 Å) of an α-helix. Spermine could provide a multiple cross-linking of (i), ($i + 4$), ($i + 7$), and ($i + 11$) residues by ionic interactions to stabilize the α-helix conformation as shown in Figure 1a. We wish to report here the synthesis of a designed peptide that adopts the α-helical conformation in the presence of spermine.

A 14-residue peptide (SBP) was designed to have four glutamic acid residues separated by two or three residues to provide a negatively charged binding site for spermine when the peptide adopts the α-helical conformation. Alanine and glutamine residues were used as neutral residues to provide an additional potential[3c] to the peptide to adopt α-helical conformation. Figure 1b shows the sequence of SBP. Amphiphilicity of the peptide was kept minimal to avoid peptide aggregations.[3a,b] A tyrosine residue was introduced at the N-terminus as a spectroscopic probe.

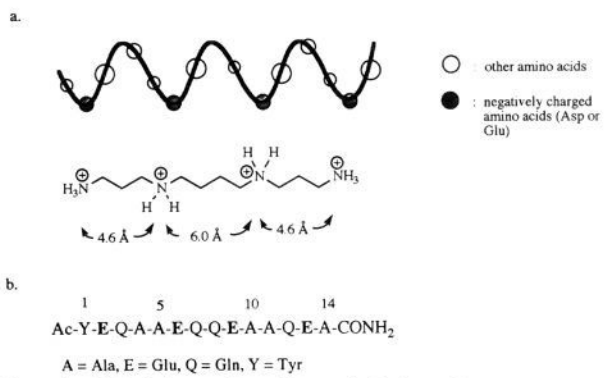

Figure 1. (a) Ionic interactions of a negatively charged α-helical peptide with fully protonated spermine in the extended conformation. (b) Sequence of a spermine-binding peptide, SBP. Glutamic acids are highlighted in the sequence with bold letters.

Experimental Section

Peptide Synthesis and Purification. All chemicals and supplies were of high purity and were available from commercial sources. The peptide, SBP, was synthesized in an Applied Biosystem (ABI-430A) peptide synthesizer using the conventional Boc solid-phase chemistry with p-methylbenzhydrylamine resin (0.72 g, 0.50 mmol, S = 0.69 mequiv/g). The peptide was acetylated with excess acetic anhydride, cleaved, and deprotected with the TMSOTf–TFA reagent[4] in the presence of thioanisole and m-cresol as scavengers. After gel filtration on Sephadex G-15 with 2% NH_4HCO_3 as an eluent, SBP was purified with reverse-phase HPLC (semipreparative Vydac C4 (300-Å pore) column). A linear gradient of acetonitrile–water–0.1% TFA was used to separate SBP from impurities. SBP eluted as a major peak at 40% acetonitrile. SBP: amino acid analysis (expected values in parentheses) Glu/Gln 7.9 (8), Ala 5.0 (5), Tyr 1.1 (1); FAB mass spectrum m/e = 1606.4, $(M + H)^+$ = 1606.7, ΔM = 0.3.

Circular Dichroism Measurements. pH was measured with a PHM84 pH meter (Radiometer). SBP concentration was initially determined by amino acid analysis (AAA) using valine as an internal standard. The extinction coefficient of SBP was then determined at λ = 272 nm (ϵ =

[†] University of Washington.
[‡] Zymogenetics.

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1.34×10^3 M^{-1} cm^{-1} in 10 mM Mes–Tris buffer, pH = 7.0). All CD spectra were recorded on a JASCO J-720 circular dichroism spectropolarimeter using a water-jacketed cylindrical cell with the path length of 0.1 or 0.5 mm. The cell temperature was kept constant with a circulating water bath either at 4 °C or at 25 °C. The α-helicity was calculated on the basis of the reported value[5] of molar ellipticity of a 100% helical peptide, $[\theta](222\ nm) = -33\ 500$ deg cm^2 $dmol^{-1}$.

Binding Constants. A lyophilized sample of SBP was dissolved in 10 mM Mes-Tris buffer, pH = 7.0. The concentration of SBP was determined by using its extinction coefficient at 272 nm. Polyamines were also dissolved in the same buffer. The stock solutions of SBP and polyamines were then mixed with an appropriate ratio. The total volume was adjusted to 110 μL with the buffer and TFE. The final concentration of TFE was 30% in 10 mM Mes–Tris buffer, pH = 7.0. A total of 9 samples with different polyamine/SBP ratios were prepared for a typical binding experiment. Water-jacketed CD cells were equipped with a constant-temperature bath. The CD spectrum of each sample was recorded from 300 through 180 nm. Each titration experiment was repeated more than three times to ensure reproducibility. Binding constants were then calculated using a nonlinear curve fitting program.

Sedimentation Equilibrium. An aqueous solution of SBP (5.1×10^{-4} M) and spermine (5.0×10^{-4} M) was prepared in 10 mM Mes buffer, pH = 6.0. The SBP–spermine complex was prepared by mixing an equal volume of the two solutions. Dextran was added to the solution (10 mg/mL) to stabilize the concentration gradient during the experiment. The solutions (150 μL) containing SBP and the SBP–spermine complex were centrifuged for 22 h using an air-driven ultracentrifuge (Beckman Airfuge) equipped with a 30° rotor. In a typical experiment, the rotor speed was 8.25×10^4 rpm, as measured by a digital tachometer. The gradient was analyzed by taking 14 aliquots of 10 μL each using a microsyringe equipped with a micromanipulator. Each aliquot was diluted with 600 μL of 10 mM Mes buffer, pH 6.0. The absorbance (A) at 272 nm of each sample was then recorded. A slope was calculated from a plot of $\ln(A)$ vs r^2, where r is the distance of each fraction from the center of the rotor. The apparent molecular weight (MW) was calculated for SBP and the SBP–spermine complex according to the published equation,[6] i.e. MW_{app}(SBP) = 1450 (MW_{calc} = 1606) and MW_{app}(SBP–spermine complex) = 1890 (MW_{calc} = 1810).

Addition of 30% TFE produced no gradient for both SBP and the SBP–spermine complex. This may be due to instability of the concentration gradient in the presence of 30% TFE. A similar experiment with cytochrome c in 30% TFE gave only 26% of the actual MW of cytochrome c (MW = 13 000).

Results

SBP was synthesized by the conventional t-Boc-based solid-phase peptide synthesis using p-methylbenzhydrylamine resin. Circular dichroism (CD) spectra of SBP showed an intense negative peak at 195 nm, characteristic of a disordered conformation, in 10 mM Mes buffer, pH 6.0 at 4 °C. The peptide becomes more α-helical (19%) in the presence of 30% trifluoroethanol (TFE) as shown in Figure 2. Titration of the peptide solution with spermine showed a significant increase in α-helicity to 38%. A curve-fitting analysis of the titration curve showed the formation of a 1:1 spermine–SBP complex[7] with the binding constant of $(2.0 \pm 0.5) \times 10^4$ M^{-1} in 10 mM Mes–Tris buffer in 30% TFE at 4 °C. Both SBP and the spermine–SBP complex were found to be monomeric in aqueous buffer solution, on the basis of the sedimentation equilibrium experiment. In 30% TFE, although the sedimentation equilibrium experiment showed no concentration gradient, the formation of aggregated SBP species is expected to become less favorable in the presence of TFE. Furthermore, the α-helicity of SBP was independent of the concentration between 3.6×10^{-5} and 3.6×10^{-4} M, suggesting the monomeric state of SBP in 30% TFE–aqueous buffer solution.

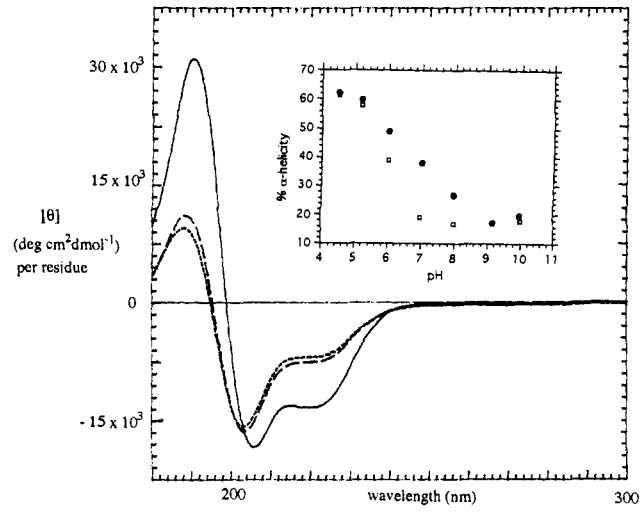

Figure 2. Circular dichroism spectra of SBP itself (···), and in the presence of equimolar amounts of ammonium chloride (- - -) and spermine (—). Spectra were obtained on a JASCO 700 CD spectrophotometer in 0.1-mm cells using 3.24×10^{-4} M solutions in 10 mM Mes–Tris buffer–30% trifluoroethanol (TFE at pH = 7.0 and 4 °C (x axis, wavelength (nm); y axis, molar ellipticity per residue (deg cm^2 $dmol^{-1}$)). Inset: pH dependence of calculated α-helicities of SBP (□) and the SBP–spermine complex (●). Acetate (pH 4.5 and 5.2), Mes (pH 6), Mes-Tris (pH 7), Tris (pH 8), and CAPSO (pH 9.2 and 10) buffers (10 mM) were used; Mes = 2-(N-morpholino)ethanesulfonic acid, Tris = tris(hydroxymethyl)-aminomethane, CAPSO = 3-(cyclohexylamino)-1-propanesulfonic acid.

Other structurally related polyamines were examined to investigate the importance of spermine structure on the stabilization of the α-helical conformation of SBP. Table I summarizes CD changes observed with SBP in the presence of various polyamines. Binding constants for spermidine and triethylenetetramine to SBP were determined to be $(9 \pm 3) \times 10^2$ and $(2.0 \pm 0.5) \times 10^2$ M^{-1}, respectively. Figure 3 shows the binding curves for these polyamines together with that for spermine. The SBP–spermine complex showed a much higher binding constant and a larger overall change in α-helicity.

Discussion

Our results strongly suggest that a stable complex is formed between fully protonated spermine and deprotonated helical SBP as shown in Figure 1a. The spermine-induced change in α-helicity of SBP showed a strong pH dependency that is consistent with the ionic nature of the interaction. The maximum change in α-helicity was observed at pH = 7 as shown in Figure 2. The decrease in $\Delta(\alpha$-helicity) at lower pH is consistent with the protonation of the glutamate side chain. Deprotonation of the spermine cation is likely to account for the decrease at higher pH. Similar pH dependencies of α-helicity have been observed for small synthetic peptides with intramolecular ion pairs between side chains.[8]

Only a slight increase in the α-helicity of SBP was observed in the presence of ammonium chloride under the same conditions, suggesting a specific interaction between spermine and SBP. Various truncated spermine analogues were examined to study the degree of specificity for the conformational change of SBP induced by spermine. Diamines, putrescine, and ethylenediamine induced a negligible change in the CD spectrum of SBP. Spermidine, a truncated analogue of spermine with three positive charges, showed only a moderate effect on the α-helicity of SBP,

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(7) Binding constants were determined at three different concentrations of SBP to see if oligomeric complexes with more than the 1:1 stoichiometry were formed. The calculated values were $(3 \pm 2) \times 10^4$, $(2.0 \pm 0.5) \times 10^4$, and $(5.2 \pm 1.6) \times 10^4$ M^{-1} for 5.90×10^{-4}, 3.24×10^{-4}, and 1.13×10^{-4} M SBP, respectively. The binding constant appears to be unchanged in spite of the large variation in the concentration of SBP, supporting the formation of the 1:1 complex.

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Table I. Molar Ellipticities of SBP at 222 nm in the Presence of Various Polyamines[a]

		SBP +					
	SBP	MIN	DIN	PUT	ETD	TET	NH_4
4 °C	6.5×10^3 [b]	13×10^3	9.8×10^3	8.0×10^3	8.0×10^3	8.1×10^3	7.1×10^3
	(19 ± 2)[c]	(38 ± 3)	(29 ± 2)	(24 ± 1)	(24 ± 2)	(24 ± 4)	(21 ± 4)
25 °C	3.8×10^3 [b]	8.1×10^3	6.0×10^3	4.8×10^3	4.8×10^3	5.2×10^3	4.7×10^3
	(11 ± 2)[c]	(24 ± 1)	(18 ± 1)	(14 ± 2)	(14 ± 2)	(15 ± 2)	(14 ± 2)

[a] MIN = spermine, DIN = spermidine, PUT = putrescine, ETD = ethylenediamine, TET = tetraethylenetetramine, and NH_4 = ammonium chloride. [b] Molar ellipticity ($[\theta](\pm 0.1) \times 10^3$) at 222 nm (deg cm^2 dmol^{-1} (per residue)). [c] Calculated α-helicity (%). Conditions: 10 mM Mes–Tris buffer – 30% trifluoroethanol, pH = 7.0; [SBP] = 3.24×10^{-4} M; [polyamines] = 3.18×10^{-4} M.

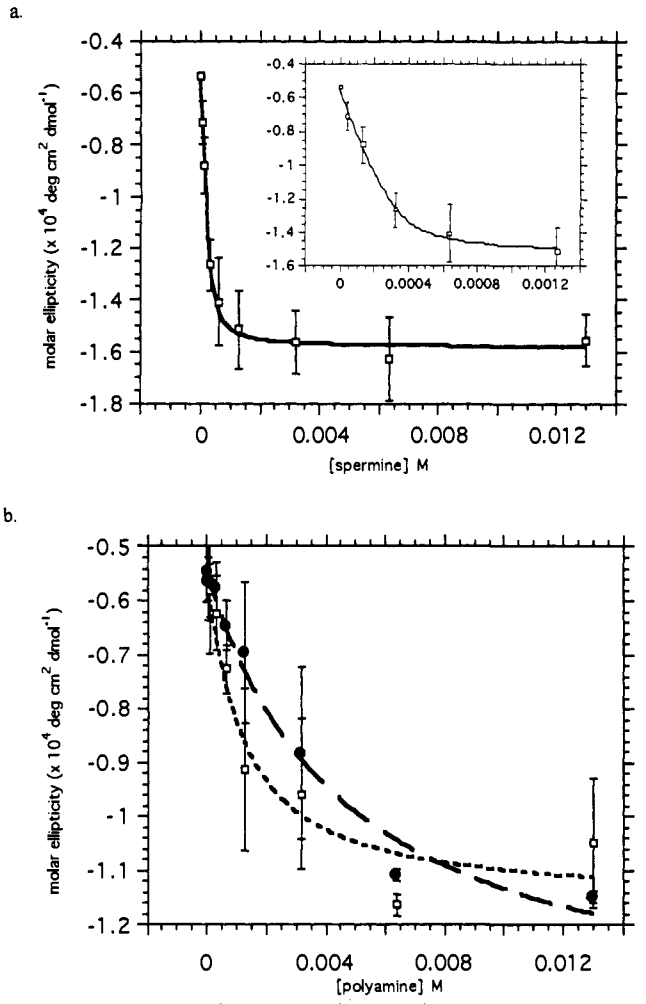

Figure 3. (a) CD titration of SBP with spermine. Inset: expansion of the binding curve at lower concentration region. (b) CD titration of SBP with spermidine (···) and triethylenetetramine (- - -). Data were fitted to the equation for a complex formation with 1:1 stoichiometry: SBP + polyamine $\overset{K}{\rightleftarrows}$ (SBP)(polyamine), K = binding constant (M^{-1}). Calculated binding constants are $(2.0 \pm 0.5) \times 10^4$, $(9 \pm 3) \times 10^2$, and $(2 \pm 0.5) \times 10^2$ M^{-1} for spermine, spermidine, and triethylenetetramine, respectively. All experiments were carried out in 30% TFE–10 mM Mes–Tris buffer, pH = 7.0. [SBP] = 3.24×10^{-4} M at 4 °C.

suggesting that four positive charges are required for the maximum conformational transition. Binding of positively charged polyamines to SBP should neutralize negatively charged glutamate residues whose repulsive interactions would destabilize the α-helical conformation. In the SBP–spermine complex, protonated spermine not only neutralizes negative charges of SBP but also could crosslink four glutamate residues to constrain the conformation of SBP in favor of the formation of the α-helical conformation as shown in Figure 1. The α-helicity of the SBP–spermine complex, however, is no more than that of the peptide alone at pH 4, where all four glutamate residues should be protonated. This observation suggests that the SBP–spermine complex may have a structural strain in the helical conformation and that the peptide motif we used may not be ideal for spermine binding. It would be interesting to examine the binding of spermine to a series of SBP-related peptides that contains four glutamate or aspartate residues at different locations in the sequence. Triethylenetetramine (TET) caused only a small increase in α-helicity of SBP. TET has four positive charges separated by only 2 carbons that should provide a great structural strain in the α-helical conformation when the complex is formed.

Polyamines are ubiquitous in nature and are known to play essential roles in various cell events.[9] It is well-known[10] that nucleic acids change their conformations in the presence of polyamines. Because of the polycationic nature of polyamines, negatively charged proteins could also interact with polyamines. Only a few proteins have, however, been known to specifically interact with spermine and its derivatives. Spermine, for example, binds to actin to facilitate the fiber formation that is essential to cell division.[11] Recently, spermine was also found in the brain and was reported to bind to the *N*-methyl-D-aspartate receptors to modulate the ion currents across the membrane.[12] Sequence analysis of proteins registered in the protein data bank[13] revealed more than 500 sequences that have a motif of E-X-X-X-E-X-X-E-X-X-X-E, where X is any amino acid. Although no helical structure with the motif was found, four unique helical sequences[14] were identified for a truncated motif, i.e. E-X-X-X-E-X-X-E, by using the relational structure data base IDITIS. It is conceivable that spermine binding to these sequences could induce a local α-helical structure that may, in turn, regulate their biological activities. Since no spermine-bound protein has been structurally characterized, model studies with synthetic oligopeptides would provide foundations to understand the mechanism of spermine–protein interactions. Synthesis and structural studies of these predicted native spermine-binding sequences are now in progress.

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(14) The sequences identified are Glu-Gly-Ile-Leu-Glu-Val-Ala-Glu (cytochrome c_{550}), Glu-Tyr-Gly-Ala-Glu-Ala-Leu-Glu (hemoglobin), Glu-His-Arg-Gln-Glu-Leu-Ile-Glu (cytochrome $P450_{CAM}$), and Glu–Glu–Leu–Leu–Glu–Leu–Val–Glu (elongation factor Tu (domain 1)).