NMR Studies on YSPTSPSY: Implications for the Design of DNA Bisintercalators

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NMR (600 MHz) studies on YSPTSPSY (1), the heptad repeat unit of RNA polymerase II with an extra tyrosine added to the N-terminus, show that the peptide is partially structured in aqueous solution. Peptide 1 contains two overlapping SPXX sequences and has been reported to bind to DNA by bisintercalation (Suzuki, M. *Nature* **1990**, *344*, 562–565). In 90% H_2O solution at pH 3.2, the major species, which is present in >90%, contains Pro^3 and Pro^6 in the trans conformation. At low temperature (4 °C), NOE connectivities are consistent with the presence of β-turn structures in equilibrium with unfolded forms of the peptide. A strong d_{NN} connectivity between Thr^4/Ser^5, a $d_{\alpha N}$ connectivity between Pro^3/Thr^4, and a medium-range NOE between Pro^3 α and Ser^5 NH indicate the presence of a β-turn formed by (i) Ser^2-Pro^3-Thr^4-Ser^5. A strong d_{NN} connectivity between Ser^7/Tyr^8 and weaker $d_{N\delta}$ NOE connectivities between Ser^2/Pro^3 δ and Ser^7/Pro^6 δ were also detected. The solution conformation of the peptide appears to have a crucial role in determining the interaction of the peptide with DNA, given that only bisintercalation has been reported in DNA-binding studies on 1 (Suzuki, M. *Nature* **1990**, *344*, 562–565). On the basis of these results, the peptide unit –SPTSPS– (3) has considerable potential as a structured linker in the preparation of DNA bisintercalators of the general structure Xaa–SPTSPS–Zaa (2) with improved selectivity properties.

DNA intercalators are an established class of anticancer drugs that exert their biological activity by disrupting the action of topoisomerase enzymes resulting in DNA strand breaks.[1] Intercalators have also been successfully employed to target interaction with DNA by acting as carriers of other reactive functionalities such as alkylating agents and have been incorporated in the design of bioreductive ligands as hypoxia selective drugs.[2] In order to develop drugs with improved selective toxicity, considerable effort has focused on the synthesis and characterization of DNA-bisintercalating agents which have a greater potential for binding to, and discriminating between, defined nucleotide sequences as a consequence of their larger binding site size.[3] Studies carried out on synthetic bisintercalators have shown that ligand rigidity plays a major role in determining sequence specificity, the mode of interaction with DNA, and antitumor activity.[3]

The naturally occurring quinoxaline antitumor antibiotics echinomycin and triostin each contain two chromophores attached to a depsipeptide ring and exert their biological activity by binding to DNA by bisintercalation.[1a,4] X-ray crystallography and NMR spectroscopic studies of these drugs and drug–DNA complexes have shown that the depsipeptide linker forms a short right-handed rectangular β-sheet that serves to orient the two intercalating quinoxaline chromophores roughly parallel and separated by distances ideal for spanning two base pairs of DNA.[3,4] On binding to DNA, these intramolecular hydrogen bonds break and are replaced by intermolecular hydrogen bonds between the cyclic peptide and the DNA base pairs.[4b] Thus the depsipeptide serves two important roles: the β-sheet hydrogen-bonding network ensures that the drug is essentially preorganized for DNA bisintercalation, i.e., is in the conformation (or very close to that) required. In addition, the peptide units, on binding to DNA, form hydrogen bonds to the DNA base pairs stabilizing the drug–DNA complex. Thus, some sequence selectivity and binding affinity is encoded for by the depsipeptide linker unit.

From a design perspective, the linker in a DNA bisintercalator should be sufficiently rigid to prevent aggregation and formation of intermolecular complexes. However, the linker requires some flexibility in order to match the dimensions of the DNA grooves which are highly sequence dependent.[5] The linker should ideally contain DNA-binding groups, both to increase the binding constant of the drug–DNA complex (although this is not a necessary condition), and also as a mechanism whereby sequence selectivity may be achieved. The vast majority of synthetic bisintercalators reported[3] incorporate flexible linkers in their structures that serve only the role of separating the

(1) (a) *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Verlag Chemie: Weinheim, 1983; pp 1–127. (b) *Chemistry of Antitumor Agents*; Wilman, D. E., Ed.; Blackie & Son Limited: Glasgow, 1990; pp 1–59; pp 403–436.

(2) See, for example: (a) Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Denny, W. A. DNA-directed Alkylating Agents. 4. 4-Anilinoquinoline-based Minor Groove Directed Aniline Mustards. *J. Med. Chem.* **1991**, *34*, 1552–1560. (b) Gordie, T. A.; Prakash, A. S.; Wakelin, L. P. G.; Woodgate, P. D.; Denny, W. A. Synthesis and Evaluation of DNA-Targeted Spatially Separated Bis(aniline mustards) as Potential Alkylating Agents with Enhanced DNA Cross-Linking Capability. *J. Med. Chem.* **1991**, *34*, 240–248. (c) Valu, K. K.; Gourdie, T. A.; Boritzki, T. J.; Gravatt, G. L.; Baguley, B. C.; Wilson, W. R.; Wakelin, L. P. G.; Woodgate, P. D.; Denny, W. A. DNA-Directed Alkylating Agents. 3. Structure-Activity Relationships for Acridine-Linked Aniline Mustards: Consequences of Varying the Length of the Linker Chain. *J. Med. Chem.* **1990**, *33*, 3014–3019. (d) Denny, W. A.; Atwell, G. J.; Anderson, R. F.; Wilson, W. R. Hypoxia-Selective Antitumor agents. 4. Relationships between Structure, Physicochemical Properties, and Hypoxia-Selective Cytotoxicity for Nitracrine Analogs with Varying Side Chains: the "Iminoacridan Hypothesis". *J. Med. Chem.* **1990**, *33*, 1288–1295.

(3) Wakelin, L. P. G. Polyfunctional DNA Intercalating Agents. *Med. Res. Revs* **1986**, *6*, 275–339 and references cited therein.

(4) (a) Ughetto, G.; Wang, A. H.-J.; Quigley, G. J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. A Comparison of the Structure of Echinomycin and Triostin A Complexed to a DNA Fragment. *Nucleic Acids Res.* **1985**, *13*, 2305–2323. (b) Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Hakoshima, T.; van der Marel, G. A.; van Boom, J. H.; Rich, A. The Molecular Structure of a DNA-Triostin A Complex. *Science* **1984**, *225*, 1115–1121. (c) Yu, C.; Yang, T.-H.; Young, J.-J. The Conformation of Echinomycin in DMSO Solution. *Biochim. Biophys. Acta* **1991**, *1075*, 141–145. (d) Arnold, E.; Clardy, J. Crystal and Molecular Structure of BBM-928 A, a Novel Antitumor Antibiotic from *Actinomadura Luzonesis*. *J. Am. Chem. Soc.* **1981**, *103*, 1243–1244.

(5) See, for example: Nadeau, J. G.; Crothers, D. M. Structural basis for DNA Bending. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2622–2626.

0022-2623/92/1835-4658$03.00/0

chromophores, and not surprisingly, binding constants have been significantly less than the values predicted.[3] An exception is ditercalinium (NSC 366241), a synthetic bisintercalator in which two ellipticene rings are connected by a rigid bisethyl piperidine chain. Ditercalinium exhibits strong antitumor properties and activates DNA repair processes.[6] Introduction of methylenes between the piperidine rings, which increases the flexibility of the linker chain, destroys the activity of the drug.[6]

This paper reports NMR studies on YSPTSPSY (1), the heptad repeat unit of RNA polymerase II with an extra tyrosine added to the N-terminus. This peptide has been reported to bind to DNA by bisintercalation.[7] NOESY experiments show that, in aqueous solution, the peptide is partially structured and there is evidence for β-turn conformations in the two overlapping SPXX subunits in peptide 1. Formation of two interlocked reverse turns disposes the two phenolic rings of the terminal tyrosine residues in the correct spacial arrangement to bind across two base pairs of DNA.[7] This result demonstrates that structured peptides may be used to prepare preorganized DNA bisintercalators of the general structure Xaa–SPTSPS–Zaa (2) where Xaa and Zaa are nonproteinogenic amino acids containing intercalator side chains.

Results

NMR spectroscopy was used to study the molecular conformation of peptide 1 in aqueous solution. While the peptide exhibits good solubility in other solvents, the present study was restricted to aqueous solutions in order to extrapolate the results to the observed properties of synthetic antitumor bisintercalators.

NMR Assignment. The NMR spectrum of the major conformation of peptide 1 was assigned using a combination of DQF-COSY,[8] HOHAHA,[9] and NOESY[10] spectra by standard methods, and the chemical shifts are summarized in Table I. Initial experiments were carried out at 400 MHz. However, the results obtained were difficult to analyze as the chemical shifts of the $H_{\beta,\beta'}$ protons in Ser^2, Ser^5, and Ser^7 were coincident with $H_{\delta,\delta'}$ in Pro^3 and Pro^6. The unambiguous assignment of the Pro $H_{\delta,\delta'}$ resonances was particularly important as these protons were crucial to assignment of any reverse turn structures (see below). In addition, due to the small size of the peptide and the magnitude of the rotational correlation time in solution,[11] only weak peaks were observed in NOESY spectra, and it was necessary to record the corresponding rotating-frame ROESY[12] spectrum to observe all the sequential connectivities. ROESY spectra suffer the disadvantage that spectral artifacts (mainly HOHAHA peaks) may appear in addition to NOE peaks and hence several experiments need to be recorded at varying field strengths to verify that peaks are indeed genuine NOE peaks.[12b] More satisfactory results were obtained at 600 MHz. The greater chemical shift dispersion allowed complete assignment of all spin systems. In addition, the higher field strength altered the rotational correlation time of the peptide 1,[11] and excellent NOESY spectra were obtained.

Table I. Chemical Shifts of 1 in ppm at 4.0 °C in 90% H_2O at pH 3.2

residue	NH	Hα	Hβ	other
Tyr^1		4.22	3.12, 3.05	7.09, 6.08
Ser^2	8.53	4.75	3.79, 3.73	
Pro^3		4.40	2.17, 1.74	1.97 (Hγ,γ'); 3.69, 3.60 (H,δ,δ')
Thr^4	8.22	4.31	4.20	1.18 (Me)
Ser^5	8.31	4.76	3.86, 3.78	
Pro^6		4.38	2.32, 1.97	2.00 (Hγ,γ'); 3.77, 3.70 (H,δ,δ')
Ser^7	8.38	4.35	3.74	
Tyr^8	8.04	4.54	3.09, 2.92	7.09, 6.08

The NMR spectrum of peptide 1 in 90% H_2O is presented in Figure 1. Given the presence of three serine residues and two prolines in the sequence a high degree of spectral overlap was observed. Inspection of both the aromatic and aliphatic regions of the spectrum show that in addition to the major species, peaks arising from minor species are also present. The minor resonances were assigned to cis–trans isomers that occur due to the two proline residues in the peptide 1. On the NMR time scale, cis–trans isomerization of the proline bond is slow and typically two sets of resonances are observed in the NMR spectrum.[13] By integration, the major isomer is present greater than 90% and was assigned to the more stable isomer in which Pro^3 and Pro^6 are in the trans conformation. This assignment was confirmed by observation of strong NOE peaks between Ser^2 α/Pro^3 δ and Ser^5 α/Pro^6 δ. If the major conformation is cis between either of these pairs of residues, then NOE peaks between Ser^2 α/Pro^3 α and Ser^5 α/Pro^6 α would be observed. Connectivities of this type were observed at low contour levels for the minor isomer(s) in solution.

Solution Conformation. The criteria for establishing the presence of reverse turns in small peptides (4–6 amino acids) by NMR spectroscopy has been discussed by Dyson

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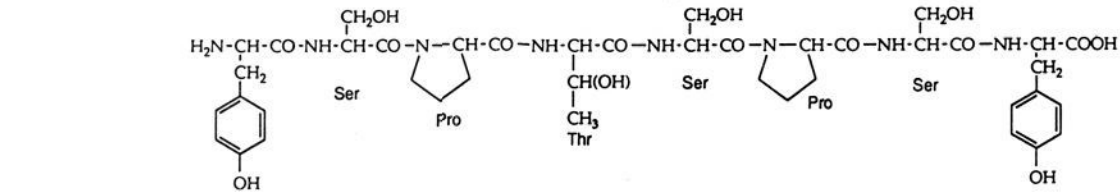

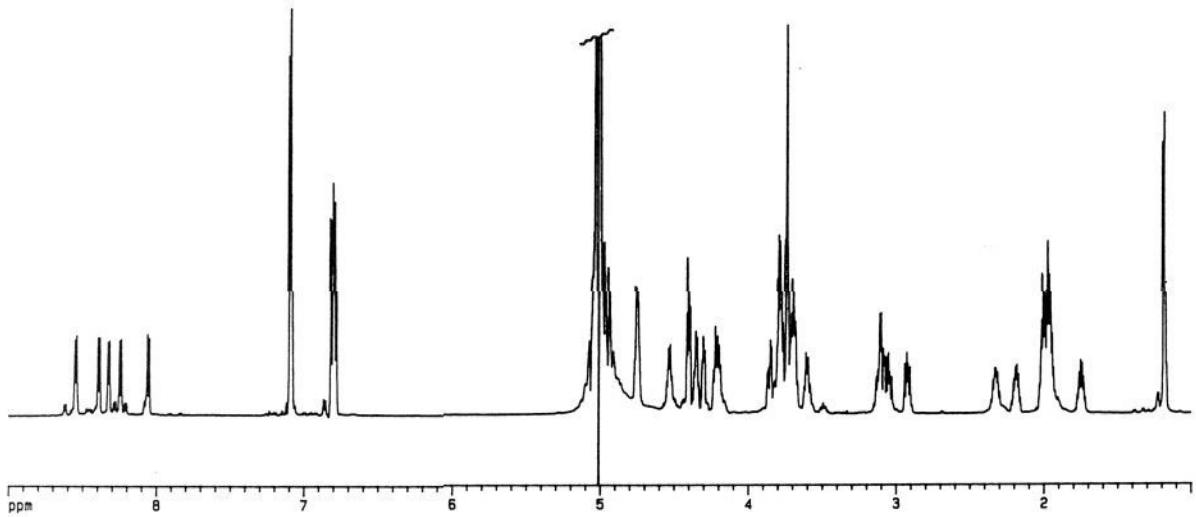

Figure 1. ^{1}H NMR (600 MHz) spectrum of **1** in 90% H_2O/D_2O at 303 K.

et al.[14] Unlike proteins, the presence of defined structure in small peptides is rare, and interpretation of the observed NMR parameters is not always straightforward as the data represent an average of the conformational ensemble present in solution.

(a) NOE Measurements. Structural information regarding the solution conformation of peptide **1** was obtained from 600-MHz NOESY experiments which were recorded at 25 and 4 °C. At room temperature, the expected sequential $d_{N\alpha}(i,i+1)$ NOE connectivities[15] were observed, which allowed unambiguous assignment of all the amino acids in the sequence.

The short-range distances that characterize type-I and type-II β-turns in short linear peptides containing proline as residue 2 are summarized in Figure 2.[14b,15] Both types of turns show strong d_{NN} connectivities between residue 3 and 4 of the turn and a weaker $d_{\alpha N}$ connectivity between residue 2 and 4. In addition to these two peaks, a type-I turn is characterized by an NOE between Pro δ and the NH of residue 3 of the turn, while in a type-II turn, the NOE is between Pro δ and the α-hydrogen of residue 3 in the turn.

Peptide **1** may be considered to consist of two overlapping SPXX sequences: (i) Ser^2-Pro^3-Thr^4-Ser^5 and (ii) Ser^5-Pro^6-Ser^7-Tyr^8. At low temperature d_{NN} NOE peaks

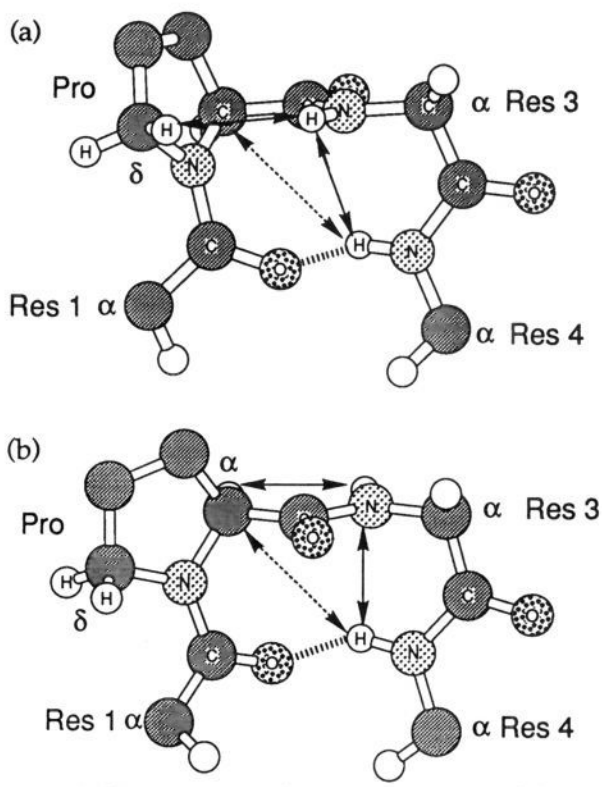

Figure 2. Short interproton distances in (a) type-I and (b) type-II turns with proline at position 2.[14b]

were observed between Ser^7/Tyr^8 and Thr^4/Ser^5 (Figure 3c). In addition, strong sequential $d_{\alpha N}$ NOE peaks, (which are also characteristic of the unfolded peptide) were observed between Pro^3 α/Thr^4 NH and Pro^6 α/Ser^7 NH. Of most significance is the medium-range $d_{\alpha N}$ NOE observed between Pro^3 α and Ser^5 NH (Figure 3b). This

(14) (a) Dyson, H. J.; Cross, K. J.; Houghten, R. A.; Wilson, I. A.; Wright, P. E.; Lerner, R. A. The Immunodominant Site of a Synthetic Immunogen has a Conformational Preference in Water for a Type-II Reverse Turn. *Nature* **1985**, *318*, 480–483. (b) Dyson, H. J.; Rance, M.; Houghton, R. A.; Lerner, R. A.; Wright, P. E. Folding of Immunogenic Peptide Fragments of Proteins in Water Solution I. Sequence Requirements for the Formation of a Reverse Turn. *J. Mol. Biol.* **1988**, *201*, 161–200. (c) Wright, P. E.; Dyson, H. J.; Lerner, R. A. Conformation of Peptide Fragments of Proteins in Aqueous Solution: Implications for Initiation of Protein Folding. *Biochemistry* **1988**, *27*, 7167–7175. (d) Dyson, H. J.; Wright, P. E. Defining Solution Conformations of Small Linear Peptides. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 519–538.

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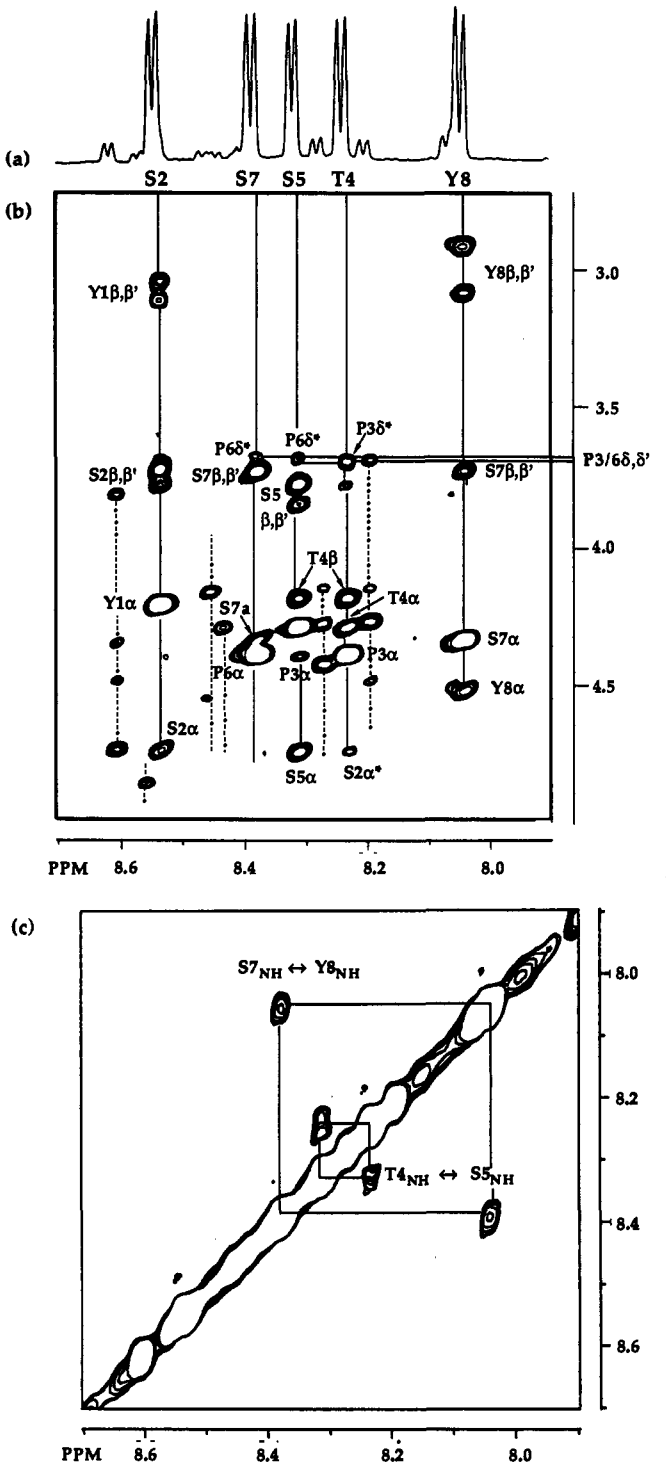

Figure 3. Portion of 600-MHz NOESY spectrum of 1 recorded with a mixing time of 500 ms at 4.0 °C: (a) resolution enhanced one-dimensional spectrum (b) fingerint region, and (c) amide region. Peak assignments refer to the spin systems of the major resonances in the spectrum. Dotted lines indicate peaks arising from the minor cis–cis and cis–trans isomers and were not assigned. Starred peaks are tentative assignments; these peaks may be due to minor isomers in solution (see text).

NOE taken together with the d_{NN} (3,4) and $d_{\alpha N}$ (2,3) NOEs strongly suggests the presence of a significant population of 1 in which (i) Ser^2-Pro^3-Thr^4-Ser^5 adopts a type-II turn (Figure 2b). The expected medium-range $d_{\alpha N}$ (2,4) NOE between Pro^3 α and Tyr^8 NH was not observed for sequence (ii) Ser^5-Pro^6-Ser^7-Tyr^8. Thus, while there is good NOE evidence for the presence of a β-turn conformation in sequence (i) Ser^2-Pro^3-Thr^4-Ser^5, sequence (ii) is significantly less structured and most probably unfolded forms predominate in this region of the peptide. With shorter mixing times and at room temperature, only sequential connectivities were observed.

The possible existence of conformations of 1 in which type I turns are present in the peptide chain cannot be ruled out. At low contour levels, peaks corresponding to NOEs between protons in the minor isomers of peptide 1 were observed (indicated by dotted lines in Figure 3b) but were not assigned for the purposes of the present study. At these low contour levels two weak NOE connectivities between Pro^3 δ/Thr^4 NH and Pro^6 δ/Ser^7 NH were tentatively assigned (starred in Figure 3b). Unambiguous assignment of these peaks was not possible as the intensity of these peaks is similar to the intensity of the NOE peaks arising from the minor species in solution. However, they are consistent with the weak NOE peaks that are expected from two type-I β-turns formed by the SPXX sequences in 1. Overall, these assignments indicate that β-turn conformations, both type I and type II are present in the sequence (i) Ser^2-Pro^3-Thr^4-Ser^5.

(b) Coupling Constants. $^3J_{N\alpha}$ Coupling constants of the five observable amide protons (Ser^2, Thr^4, Ser^5, Ser^7, Tyr^8) were measured from 1D spectra and lay in the range 7.1–8.0 Hz at 25 °C and between 6.7–7.9 Hz at 4 °C. These values are midway between the value calculated for a reverse turn (5.0 Hz) and a β-sheet structure or random coil structure (9.0 Hz) and are similar to the values observed by Dyson et al.[14b] in short structured peptides.

(c) Temperature Dependence of Amide Protons. Variable-temperature analysis of the amide region of the spectrum of 1 in the range 4–65 °C showed the expected inherent variation of chemical shift of the amide resonances with temperature. While the amide resonances of Ser^5, Thr^4, Ser^7, and Tyr^8 remained as sharp resolved doublets in this temperature range, the signal due to Ser^2 broadened appreciably and decreased in intensity at temperatures above 35 °C. This behavior is most likely a result of the close proximity of the amide proton of Ser^2 to the N-terminal amino group of the peptide. Similar results have been reported in previous studies on short peptides.[14] Measurement of the temperature coefficients in this temperature range gave values in the range of (5.7–7.1) × 10^{-3} ppm/K. In general, hydrogen bonding reduces the solvent accessibility of a proton to exchange with water and temperature coefficients of (3–4) × 10^{-3} ppm/K have been observed.[14,16]

Discussion

The two phenolic rings of the terminal tyrosines in 1 have been shown to simultaneously intercalate into DNA by fluorescence measurements.[7] To rationalize the fact that no monointercalation of peptide 1 was detected, Suzuki proposed that the peptide mimics the solution conformation of echinomycin,[4c] adopting a conformation in which the two aromatic rings of the terminal tyrosine rings are parallel and separated by a distance similar to that of the quinoxaline rings in echinomycin.

The heptad repeat unit 1 may be considered to contain two overlapping SPXX sequences:[7] (i) Ser^2-Pro^3-Thr^4-Ser^5 and (ii) Ser^5-Pro^6-Ser^7-Tyr^8. The sequence SPXX has the potential to form type-I and type-II turns and

(16) Deslauriers, R.; Smith, I. C. P. In *Biological Magnetic Resonance*; Berliner, L., Reuben, J., Eds.; Plenum: New York, 1980; Vol. 2, pp 243–335.

both types of turns have been observed in these units located in protein structures.[17] Formation of a hydrogen bond between the serine side-chain oxygen atom of residue 1 and the amide hydrogen of residue 3, in addition to the standard hydrogen bond formed between the carbonyl oxygen atom of residue 1 and the amide NH of residue 4, has been suggested to particularly stabilize a type-I turn in SPXX sequences[17] and led Suzuki to propose that the peptide 1 forms two interlocked type-I turns.

Most peptides of length 6–8 amino acids are essentially structureless in aqueous solution. Evidence that short peptides can in fact form distinct, significantly populated conformations in solution has been shown in recent years by NMR spectroscopic studies.[14,18] Clear evidence that peptide 1 is not a random coil structure in aqueous solution was obtained in NOESY experiments at low temperature. The presence of sequential d_{NN} NOE connectivities immediately identifies a significant population of non-random folded structures in the conformational ensemble. The connectivity patterns observed in the NOESY spectra of 1 are consistent with the presence of a significant population of a conformation in which (i) Ser^2-Pro^3-Thr^4-Ser^5 forms both type-I and type-II β-turn conformations. In the conformation proposed by Suzuki, (i.e., both SPXX sequences form type-I β-turns), there is one degree of rotational freedom about the central Ser^5 NH–CH$_\alpha$ bond which determines whether the tyrosine rings are on the same side of the backbone or on opposite sides.[7]

The temperature coefficients of the amide protons are slightly diminished compared to the expected values for structureless peptides. Thus, while NOE connectivities indicate the presence of partially folded forms of the peptide, these conformations are certainly in equilibrium with other random coil or partially structured conformations, with the result that larger changes in the temperature coefficients are not observed. Conformational averaging is also reflected in the values of the $^3J_{N\alpha}$ coupling constants.

The importance of preorganization of the chromophores in DNA bisintercalators is evident from the structure of natural antitumor drugs[1] and from studies of linker flexibility and constitution in synthetic compounds.[1,3] The previously reported DNA binding studies of 1 showed that the peptide binds the DNA by bisintercalation;[7] no evidence for any monointercalation was detected as would be expected for a flexible bisintercalator. Thus, while the population of the major conformation of 1 is unable to be quantified with the present data, the solution conformation of 1 appears to play a significant role in the DNA recognition process measured by Suzuki.[7] While DNA almost certainly plays a role in providing a template which stabilizes the conformation of the peptide, it is significant that the peptide is structured prior to interaction with DNA. Peptides have been frequently used to chemically link intercalating chromophores, due to their presence in natural drugs, solubility properties, and potential to form hydrogen bonds with the DNA base pairs. However, all peptide-linked bisintercalators previously reported have involved unstructured, "random coil" peptide units to link the chromophores.[3,19] In contrast, the present study shows that by appropriate choice of amino acids, structured or partially structured peptide linkers may be designed and incorporated into the structure of DNA bisintercalators.

Figure 4a shows 3D representations of the peptide 1 built into a conformation with two reverse turn structures and with the two aromatic rings oriented parallel by rotation about the central Ser^5 N–CH$_\alpha$ bond.[7] As noted by Suzuki,[7] this conformation has distinct structural similarities to the conformation of the known DNA bisintercalators echinomycin and triostin (Figure 4b). Both triostin and echinomycin have been crystallized with oligonucleotide sequences.[4a,b] The quinoxaline chromophores span two base pairs and insert themselves across d(CpG) base pairs. The potential of peptide 1 to bind to the minor groove in a similar manner was investigated by molecular modeling. The peptide 1 was built into two overlapping reverse turns, docked into the minor groove of a DNA hexamer d(TACGTA)$_2$, with the aromatic side chains sandwiching the central CG base pair, and energy minimized. The resultant complex (Figure 5) has the peptide in a similar conformation to that depicted in Figure 4a, with the linker –SPTSPS– curved around the minor groove. The linker amino acids have the potential to form hydrogen bonds to the GC base pairs, but in the absence of data on the specificity of the peptide for particular base-pair sequences, the presence of such interactions can only be speculative. The proposed binding to the minor groove is consistent with fluorescence measurements that indicate bisintercalation with DNA,[7] but clearly further experimental data is required to establish the exact structure of the DNA–peptide complex.

On the basis of the heptad unit 1, the general sequence Xaa–SPTSPS–Zaa (2), where Tyr^1 and Tyr^8 are replaced with nonproteinogenic amino acids Xaa and Zaa, provides a framework that allows two intercalators to be separated by the optimal distance for DNA bisintercalation. Thus the sequence –SPTSPS– (3) may be considered an excellent linker for the preparation of DNA bisintercalators. The key amino acids in this linker appear to be the first two residues in each turn, i.e., serine and proline. From the known occurrence of SPXX sequences,[17] generally, polar residues are tolerated at positions 3 and 4 (XX in this sequence). Hence, it is highly likely that the same β-turn conformations will occur with residues other than serine and threonine at position X.

In addition to serving the role of a structural linker, 2 has the important property of containing potential DNA-binding units. The sequence SPXX has been proposed to be a DNA recognition element, due to its high frequency in gene regulatory proteins.[17b] While there is no experimental data available to date concerning SPXX–DNA interactions, the present data[17b] indicates that the SPXX unit, when incorporated into a DNA-bisintercalator may serve to stabilize the DNA-bisintercalator complex or stabilize a DNA bisintercalator–protein complex. One of the central problems with the development of clinically useful antitumor intercalators and bisintercalators is poor

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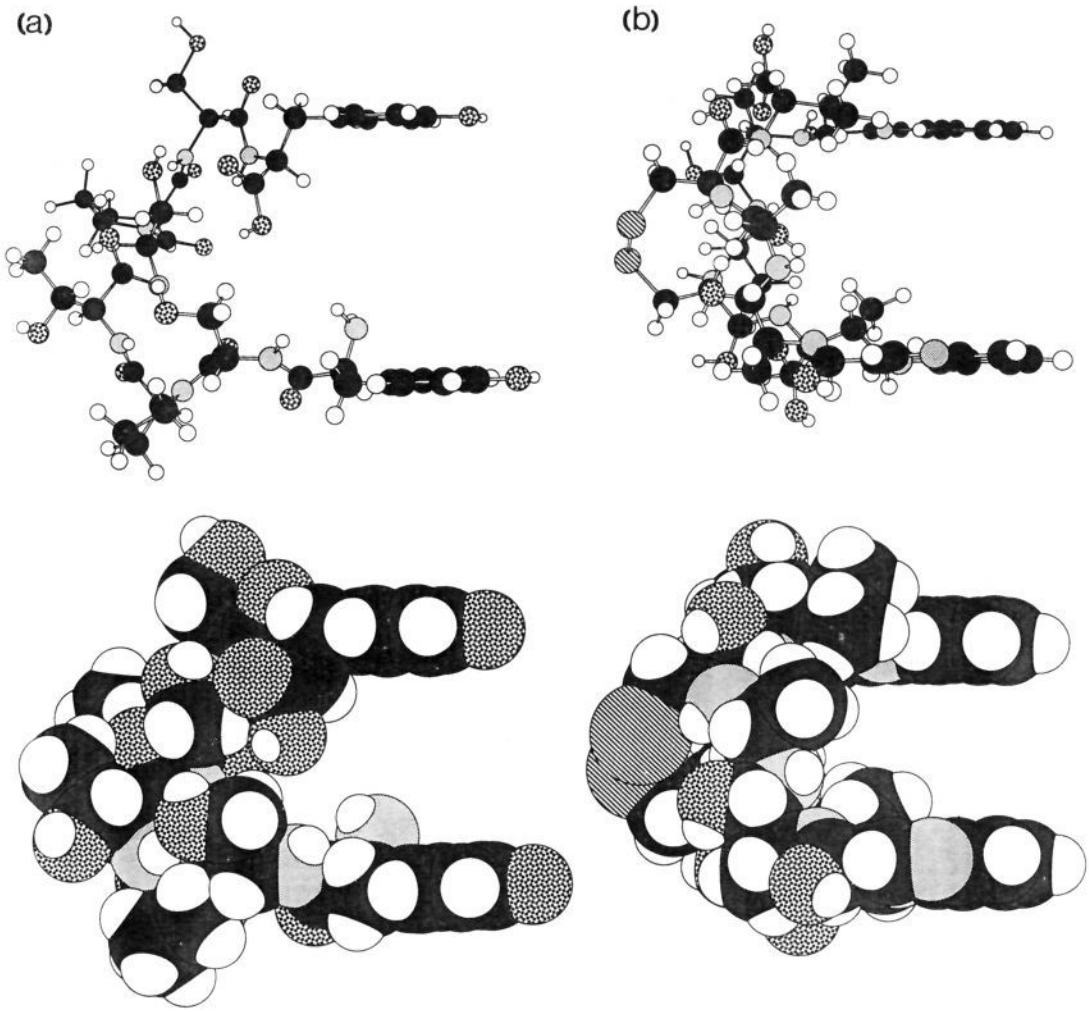

Figure 4. Stick and CPK representations of (a) peptide 1 with Ser[2]-Pro[3]-Thr[4]-Ser[5] and Ser[5]-Pro[6]-Ser[7]-Tyr[8] built into reverse turns[7] and (b) the crystal structure of the DNA-bisintercalator triostin.[4a]

selectivity resulting in toxicity and side effects.[1] The Xaa–SPTSPS–Zaa (2) sequence has the potential to achieve selectivity by interaction with DNA through the SPXX amino acids in the linker. Furthermore, incorporation of different intercalators with specific base-pair preferences at positions 1 and 8 (amino acids Xaa and Zaa) provides a mechanism whereby distinct sequence preferences may be achieved. The preparation of peptides sequences Xaa–SPTSPS–Zaa (2) is currently underway. NMR studies and biological testing results will be reported in due course.

Conclusions

NMR spectroscopy has been used to study the conformation of YSPTSPSY (1), the heptad repeat unit of RNA polymerase II with an extra tyrosine added to the N-terminus. Unlike typical peptides of this size, NOESY spectra indicate that the peptide is partially structured in solution. The data is consistent with the presence of a significant population of a conformation in which β-turn structures are present in the peptide. On the basis of these results, a new class of DNA bisintercalators may be designed with the general structure Xaa–SPTSPS–Zaa, which incorporate the structured peptide linker –SPTSPS– (3). This system has considerable advantages over previously reported peptide-linked DNA bisintercalators, as (i) the peptide is structured and hence 1 is partially preorganized for DNA bisintercalation, (ii) the linker has the potential to stabilize the DNA-bisintercalator complex as it contains SPXX units, proposed DNA recognition sequences, and (iii) introduction of different chromophores into Xaa and Zaa provides a mechanism whereby sequence selectivity may be achieved.

Experimental Section

Materials. Peptide 1 was purchased from AUSPEP Australia Ltd, Melbourne, Australia. The termini of the peptide are unblocked (see Figure 1).

NMR Spectroscopy. NMR experiments were carried out on a 40 mM solution of 1 in 90% H_2O/10% D_2O (pH 3.2) with dioxan as internal reference. NMR spectra were recorded on Bruker AMX400 and AMX600 spectrometers. The temperature was calibrated by the shift difference in methanol.[20] Spectra were recorded over spectral widths of 4000–6000 Hz with quadrature detection employed throughout. Two-dimensional spectra were acquired in the phase-sensitive mode using time-proportional phase incrementation (TPPI).[21] Data sets resulting from 400–512 increments of t_1 were acquired and zero filled to 1024 points,

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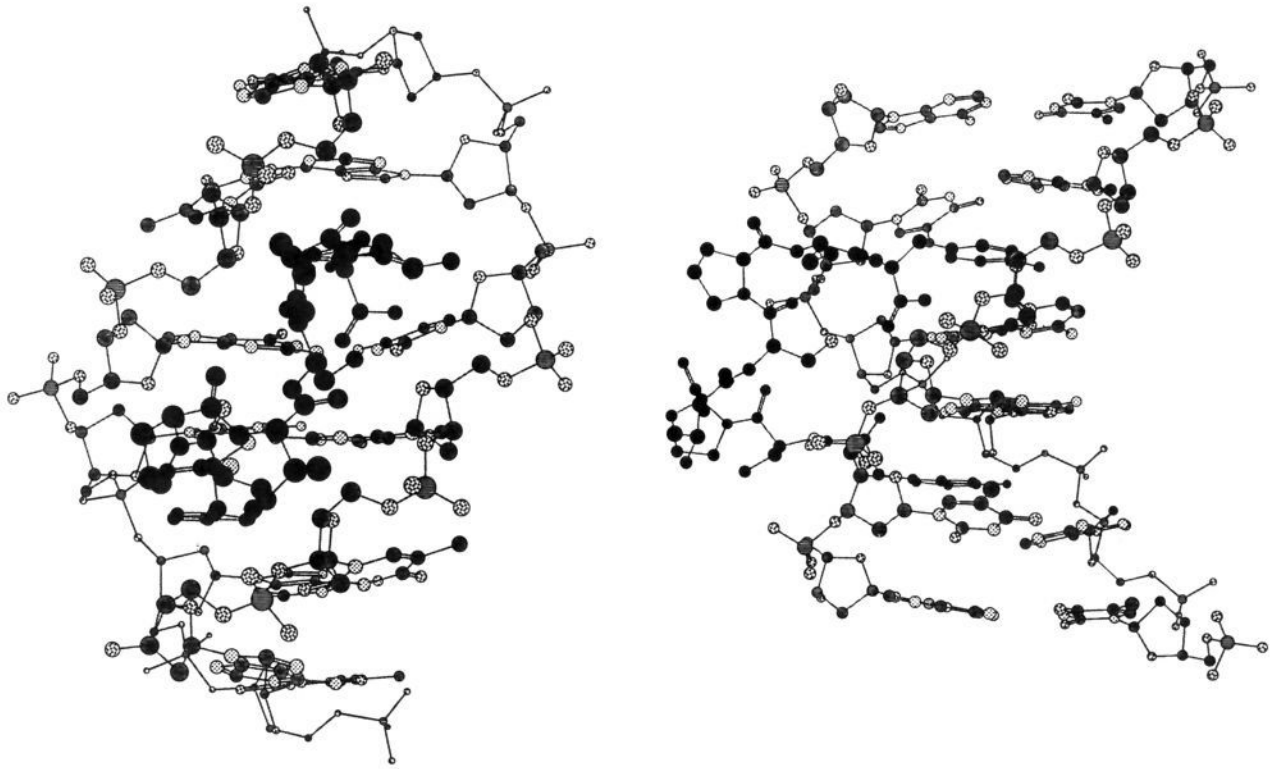

Figure 5. Peptide 1 docked into the minor groove of the hexamer d(TACGAT)$_2$ with the two aromatic residues intercalated on either side of the central CpG step. The peptide backbone is colored black. Hydrogen atoms are omitted for clarity.

with each free induction decay composed of 2048 data points. Typically 64 transients were recorded for each increment of t_1 with a recycle delay of 1.3–1.5 seconds. All spectra were recorded with the carrier placed on the ^1H_2O resonance with solvent suppression achieved by coherent presaturation during the recycle delay. Double quantum filtered COSY (DQF-COSY)[8] spectra were acquired using the standard pulse sequence. HOHAHA[9] spectra were acquired using a 8–9-kHz spin-locking field with an MLEV-17 sequence of 45–65 ms. ROESY[12] spectra were recorded using a 3.2–4.5-kHz spin-locking field and mixing times of 200, 300, and 400 ms. NOESY[10] spectra were recorded with solvent saturation during the mixing times (150, 250, and 500 ms) as well as the recycle delay. Data was subjected to shifted sine-bell weighting functions in f1 and f2 and were base line corrected where required using Bruker software on an X32 data station.

Molecular Modeling. Modeling was carried out using MacroModel[22] (version 3.1X) on a Silicon Graphics 4D/30 personal workstation. A DNA hexamer d(TpApCpGpTpAp)$_2$ was generated in a B conformation using the NUCLEIC ACIDS subroutine of MacroModel. The peptide 1 was built into the proposed conformation in which the two Ser-Pro-X-X sequences form β-turns, and the tyrosine residues are approximately parallel. This conformation was manually docked into the minor groove of the DNA with the phenolic rings sandwiching the central GC base pairs. The peptide DNA complex was subjected to approximately 2500 iterations using the Polak Ribiere conjugate gradient (PRCG) minimization algorithms and the AMBER force field resident within MacroModel.

Acknowledgment. Financial support from the Sydney University Cancer Research Fund is gratefully acknowledged.

Registry No. 1, 127959-11-9.

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