Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 651–654

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

Anion Recognition by Thiostrepton

Carolina Godoy-Alcántar,[a] Ismael León Rivera[a] and Anatoly K. Yatsimirsky[b,*]

[a]Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, 62210 Cuernavaca, Morelos, Mexico
[b]Facultad de Química, Universidad Nacional Autónoma de México, 04510 Mexico D.F., Mexico

Received 13 September 2000; accepted 27 December 2000

Abstract—A bicyclic polypeptide antibiotic thiostrepton forms both 1:1 and 1:2 complexes with anions (as tetrabutylammonium salts) in organic solvents with $K_2 > K_1$ for F^- and $K_2 \ll K_1$ for all other anions studied. Relative stabilities of 1:1 complexes in DMSO are $AcO^- \sim F^- \gg Cl^-$, Br^-, HSO_4^-, $H_2PO_4^-$, but in $CHCl_3$ they follow a different order: $Cl^- \sim HSO_4^- > F^- \sim AcO^- > Br^- > H_2PO_4^-$. The binding mode of anions to thiostrepton is discussed on the basis of solvent effects on the complexation selectivity. © 2001 Elsevier Science Ltd. All rights reserved.

Molecular recognition of anions by synthetic receptors has attracted considerable interest.[1] Particularly important are neutral hydrogen bonding receptors,[1d,2,3] which show higher selectivities, although they operate only in apolar noncompeting media. Surprisingly little attention has been paid to polypeptides as neutral anion receptors, although they possess the required structural elements. Few reported studies involve binding of 4-nitrophenyl phosphate[4] and ditopic simultaneous complexation of cations and anions[5] by synthetic cyclic hexapeptides in DMSO. At the same time, phosphate- and sulfate-binding proteins are known to bind anions strongly and selectively via neutral proton-donor groups, the majority of which are main chain protein NH groups.[6a,b] Also, a glycopeptide antibiotic vancomycin has an anion binding site constructed from peptide NH groups.[6c] This paper reports the anion complexation properties of a natural bicyclic polypeptide antibiotic thiostrepton (**1**),[7] which contains a large number of potential anion binding sites involving nine peptide NH and five aliphatic OH groups. Thiostrepton binds very tightly to ribosomes and inhibits protein biosynthesis via not yet clearly understood mechanism(s).[7b] The detection of the anion binding ability of thiostrepton may be important for better understanding of its interactions with ribosome components bearing a large number of anionic groups.

Interactions of **1** with Bu_4NX salts were studied first by ^1H NMR spectroscopy in DMSO-d_6. Sharp resonances for the NH protons of **1** broaden into the baseline on addition of Bu_4NF (Fig. 1). Addition of Bu_4NF causes considerable upfield shifts with broadening of the signals at δ 4.609 and 6.26, which can be attributed to 1-CH ($\Delta\delta = -0.147$ ppm) and 3-CH ($\Delta\delta = -0.084$ ppm), respectively, and a downfield shift of the signal at δ 6.82, which is attributed to 4-CH ($\Delta\delta = 0.126$ ppm) in the quinaldic precursor in **1**. Upfield shifts in the presence of added anions are typically observed for CH protons adjacent to hydrogen-bonded O–H[3e,f] and NH[2a] groups. It seems, therefore, that the 1-C-OH group of the quinaldic precursor is involved in F^- binding together with the peptide N–H groups. Under similar conditions, addition of Bu_4NX ($X = Cl$, Br, HSO_4) caused small, less than 0.02 ppm, upfield shifts. Larger upfield shifts were also observed with $Bu_4NH_2PO_4$: $\Delta\delta = -0.08$ ppm for CH_2 protons of the first dehydroalanine (at δ5.62) in the side chain of **1** and $\Delta\delta = -(0.05–0.1)$ ppm for unidentified signals between 4.6 and 5.6 ppm.

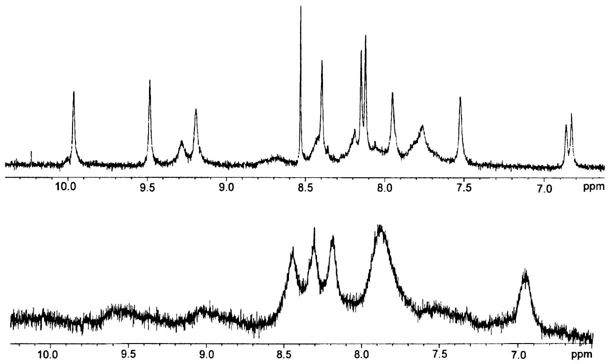


Figure 1. ^1H NMR spectra of 0.00362 M **1** (top) before and (bottom) after addition of 0.01 M Bu_4NF in DMSO-d_6.

---

*Corresponding author. Fax: +1-525-616-2010; e-mail: anatoli@servidor.unam.mx

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00025-7

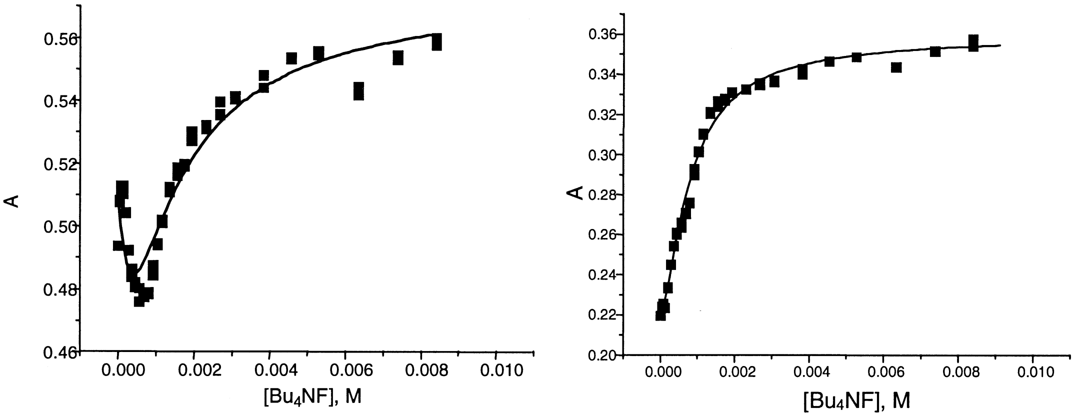
The above results indicate a possible stronger binding of fluoride and dihydrogen phosphate anions to **1**. In $CDCl_3$, solvent addition of all employed Bu_4NX salts caused noticeable characteristic downfield shifts[2a,b,3a,e] of the peptide N–H groups, which indicates stronger anion complexation in this less polar medium. Attempts to use the ^1H NMR titration method for quantitative study of anion complexation with **1** were unsuccessful, because in the presence of Bu_4NX salts the spectra were poorly resolved in both solvents. Determination of binding constants appeared to be possible by the UV titration of **1**. In preliminary experiments, the thiostrepton absorbance was measured as a function of its concentration in the range 0.005–0.1 mM in DMSO and $CHCl_3$. In the former solvent, the perfect proportionality was observed, but in $CHCl_3$ the plot was curved at concentrations above 0.02 mM indicating significant self-aggregation of thiostrepton in this solvent. All titration experiments were performed therefore with ca. 0.01 mM thiostrepton in both solvents. Addition of Bu_4NX salts induced a red shift of the absorption shoulder of **1** in the range 260–300 nm. As in ^1H NMR spectra, the largest spectral shift in DMSO was observed for Bu_4NF. Also, a significant shift was observed with Bu_4NOAc, but additions of Bu_4NX when $X^- = Cl^-$, Br^-, HSO_4^-, and $H_2PO_4^-$ did not cause detectable spectral changes. In $CHCl_3$, the complexation-induced spectral shifts were similar for all salts.

The shape of titration plots was dependent on the wavelength used. As an example, Figures 2a and b show titration plots for Bu_4NF in DMSO at two different wavelengths. Such behavior clearly indicates formation of more than one host–guest complex. In particular, a minimum in the plot in Figure 2a indicates the successive binding of two anions: the first inducing a decrease and the second inducing an increase in the absorbance of **1**. The titration plot for Bu_4NOAc (Fig. 3) shows a rapid initial increase in the absorbance at $[Bu_4NOAc] < 0.001$ M followed by a slower dependence, again indicating the successive binding of two anions. The titration plot for Bu_4NF in $CHCl_3$ (Fig. 4) has a 'sigmoid' shape that is typical for a cooperative binding of two species. Titration plots for other salts in $CHCl_3$ were similar to that for Bu_4NOAc in DMSO. All titration plots have been fitted satisfactorily to eq 1 derived for the case when both 1:1 and 1:2 host:guest binding are observed and $[\text{guest}] \gg [\mathbf{1}]$:[8]

$$A = \frac{(\varepsilon_0 + \varepsilon_1 K_1[\text{guest}] + \varepsilon_2 K_1 K_2[\text{guest}]^2)[\mathbf{1}]_t}{1 + K_1[\text{guest}] + K_1 K_2[\text{guest}]^2} \qquad (1)$$

where A is the absorbance, ε_0, ε_1 and ε_2 are molar absorptivities of free **1** and its 1:1 and 1:2 complexes, respectively, K_1 and K_2 are stepwise formation constants, and $[\mathbf{1}]_t$ is the total concentration of **1**. The values of K_1 and K_2 were calculated from the fitting of titration plots at several wavelengths to eq 1 using nonlinear least-squares regression implemented with Origin 3.5 program and averaged; the mean values are given in Table 1.

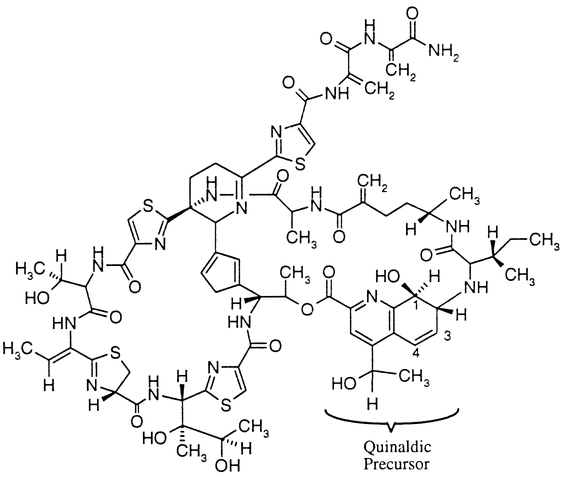
Binding of more than one anion to **1** is not unexpected taking into account large numbers of potential hydrogen bond donor groups in the host. The cooperative binding of two F^-, while for all other anions $K_2 \ll K_1$, probably is due to a complexation-induced conformation change of the host in the presence of F^-. The opposite solvent effects on K_1 and K_2 on going from DMSO to $CHCl_3$ (Table 1) are consistent with intracavity binding of the first anion (see below), but the second anion probably binds to the more exposed hydrogen bond donor groups of **1**. Comparison of binding constants of all anions studied shows the following order of affinities for the first bound anion in DMSO: $AcO^- \sim F^- \gg Cl^-, Br^-, HSO_4^-, H_2PO_4^-$. Binding of Cl^-, Br^-, HSO_4^- and $H_2PO_4^-$ may be undetectable simply due to too small complexation-induced spectral changes in the UV region and the above NMR results indicate that this may be the case for $H_2PO_4^-$, although Cl^-, Br^-, and HSO_4^- anions do not induce changes both in UV and NMR spectra and most probably have really very low affinities to **1** in DMSO.



a b

Figure 2. Titration plots for **1** and Bu_4NF at (a) 261 and (b) 300 nm in DMSO. In this and other figures, solid curves are theoretical profiles calculated from eq 1 and the binding constants given in Table 1.

Table 1. Binding constants (M^{-1}) of anions to **1** calculated from UV titration data



1

Anion	K_1, in DMSO	K_2, in DMSO	K_1, in $CHCl_3$	K_2, in $CHCl_3$
F^-	1.1×10^3 (±200)	1.9×10^3 (±300)	700 (±100)	5.0×10^3 (±900)
Cl^-	—[a]		5.2×10^3 (±200)	< 10
Br^-	—[a]		180 (±20)	—[b]
AcO^-	1.6×10^3 (±100)	17 (±10)	550 (±80)	< 10
HSO_4^-	—[a]		5.7×10^3 (±1500)	—[b]
$H_2PO_4^-$	—[a]		100 (±20)	—[b]

[a] Undetectable spectral changes.
[b] Very small K_2 value.

Binding affinities in $CHCl_3$ essentially follow a different order: $Cl^- \sim HSO_4^- > F^- \sim AcO^- > Br^- > H_2PO_4^-$. Thus, while the affinity in DMSO correlates, as expected, with anion basicity, affinity in $CHCl_3$ shows a more complex trend. This solvent, as compared to DMSO has much lower hydrogen-bond basicity, but higher hydrogen-bond acidity as follows from the respective α and β parameters:[9] for DMSO $\alpha = 0$ and $\beta = 0.76$, for $CHCl_3$ $\alpha = 0.2$ and $\beta = 0.1$.[9a] Therefore, a strong increase in K_1 for acidic HSO_4^- reflects its binding as a hydrogen bond donor and decrease in K_1 for most basic F^- and AcO^- as compared to Cl^-, Br^-, and $H_2PO_4^-$ probably reflects stronger solvation of the former anions by chloroform.[10] Interestingly, not only relative but even absolute values of K_1 for F^- and AcO^- are smaller in $CHCl_3$ than in DMSO indicating a larger importance of anion stabilization with $CHCl_3$ than solvation of hydrogen-bond donor sites in **1** with DMSO. In the case of intracavity anion binding such a situation is possible because donor binding sites of **1** turned inside the macrocycle cavity should be partially hidden from the solvent. Solvent-dependent selectivity of anion recognition by **1** certainly exemplifies a general phenomenon, which requires detailed study of solvent effects on anion binding for its interpretation.[11] Such a study with **1** is now in progress.

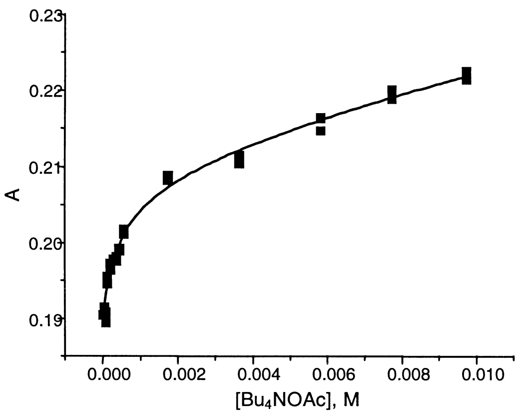


Figure 3. Titration plot for **1** and Bu_4NOAc at 300 nm in DMSO.

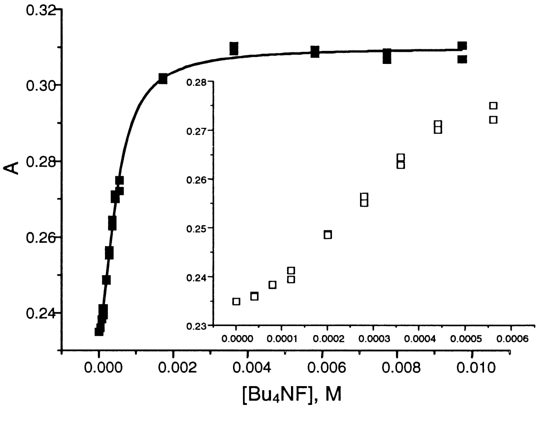


Figure 4. Titration plot for **1** and Bu_4NF at 306 nm in $CHCl_3$ (insert graph shows the region of low guest concentrations where the 'sigmoid' shape of the plot is more clearly seen).

In conclusion, we would like to discuss briefly a possible structure of anion complexes. The crystal structure of thiostrepton is incomplete,[7c] but shows that two large cycles are folded over one another creating a globular structure maintained by intramolecular hydrogen bonding. Probably anions are intercalated between the cycles and are bound to the intracavity hydrogen donor groups. In addition, a large number of electron-acceptor dehydroalanine and dehydrobutirine groups in thiostreptone should make possible a significant contribution of charge-transfer type interactions with anions.

Acknowledgements

We thank the CONACyT for support provided through the Project 3554P-E9607.

References and Notes

1. (a) For reviews, see: *The Supramolecular Chemistry of Anions*; Bianchi, A., Bowman-James, K., Garcia-España, E., Eds.; Wiley: New York, 1997. (b) Scheerder, J.; Engbersen, F. J.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays-Bas* **1996**, *115*, 307. (c) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (d) Antonisse, M. M. G.; Reinhoudt, D. N. *J. Chem. Soc., Chem. Commun.* **1998**, 443. (e) Beer, P. D. *J. Chem. Soc., Chem. Commun.* **1996**, 689.

2. (a) Werner, F.; Schneider, H.-J. *Helv. Chim. Acta* **2000**, *83*, 465. (b) Xie, H.; Yi, S.; Wu, S. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2751. (c) Jagessar, R. C.; Burns, D. H. *J. Chem. Soc., Chem. Commun.* **1997**, 1685. (d) Raposo, C.; Almaraz, M.; Martín, M.; Weinrich, V.; Mussóns, M. L.; Alcázar, V.; Caballero, M. C.; Morán, J. R. *Chem. Lett.* **1995**, 759. (e) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 369. (f) Wilcox, C. S.; Kim, E.; Romano, D.; Kuo, L. H.; Burt, A. L.; Curran, D. P. *Tetrahedron* **1995**, *51*, 621. (g) Hamann, B. C.; Branda, N. R.; Rebek, J., Jr. *Tetrahedron Lett.* **1993**, *34*, 6873.

3. (a) Kavallieratos, K.; Bertao, C. M.; Crabtree, R. H. *J. Org. Chem.* **1999**, *64*, 1675. (b) Gale, P. A.; Sessler, J. L.; Allen, W. E.; Tvermoes, N. A.; Linch, V. *J. Chem. Soc., Chem. Commun.* **1997**, 665. (c) Hüber, G. M.; Gläser, J.; Seel, Ch.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 383. (d) Valiyaveettil, S.; Engbersen, J. F. J.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 900. (e) Davis, A. P.; Gilmer, J. F.; Perry, J. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1312. (f) Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778.

4. Ishida, H.; Suga, M.; Donowaki, K.; Ohkubo, K. *J. Org. Chem.* **1995**, *60*, 5374.

5. Kubik, S.; Goddard, R. *J. Org. Chem.* **1999**, *64*, 9475.

6. (a) Leucke, H.; Quiocho, F. A. *Nature* **1990**, *347*, 402. (b) He, J. J.; Quiocho, F. A. *Science* **1991**, *251*, 1479. (c) Williams, D. H. *Aldrichim. Acta* **1991**, *24*, 71.

7. (a) Florey, K. In *Analytical Profiles of Drug Substances*; Academic: New York, 1978; Vol. 7, pp 423–444. (b) Chiu, M. L.; Folcher, M.; Griffin, P.; Holt, T.; Klatt, T.; Thompson, C. J. *Biochemistry* **1996**, *35*, 2332. (c) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 223.

8. Connors, K. A. *Binding Constants*; Wiley: New York, 1987.

9. (a) Marcus, Y. *Chem. Soc. Rev.* **1993**, 409. (b) Abraham, M. H. *Chem. Soc. Rev.* **1993**, 73.

10. We did not find experimental anion solvation free energies in $CHCl_3$, but using a correlation equation $\Delta G_{transfer}(anion) = A_{\alpha}(\Delta\alpha) + A_{\pi}(\Delta\pi^*) + A_V(\Delta V/100)$ (Markus, Y.; Kamlet, M. J.; Taft, R. W. *J. Phys. Chem.* **1988**, *92*, 3613) with parameters given in the cited reference we obtained the transfer free energies from DMSO to $CHCl_3$ for F^-, Cl^-, Br^- and AcO^- equaling −14.4, +1.4, +2.9, and −0.7 kJ/mol, respectively, in accordance with proposed stabilization of more basic anions in chloroform.

11. Different degrees of self-association of R_4NX salts in different solvents also may contribute to selectivity changes although this effect hardly can be dominant.