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Selective Recognition of Sulfate Anions by a Cyclopeptide-Derived Receptor in Aqueous Phosphate Buffer

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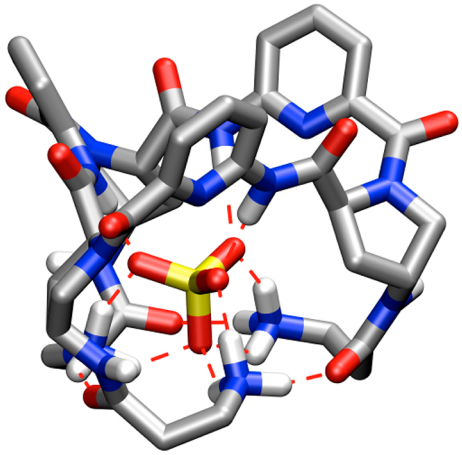
ABSTRACT



A cyclopeptide-based anion receptor containing alternating 6-aminopicolinic acid and substituted (4*R*)-4-aminoproline subunits with appended β-alanine residues binds sulfate anions in water. Importantly, appreciable sulfate binding is even observed in phosphate buffer, hence in the presence of anions of similar structure but with a different degree of protonation. The cause for the high selectivity of this receptor is related to the mode of action of the sulfate-binding protein.

Sulfate and phosphate anions are tetrahedral oxoanions with very similar ionic radii. They are associated with acid rain[1] and the eutrophications of rivers or lakes[2] and phosphate anions, including the corresponding esters and anhydrides, also have significant biological implications. The major difference between both anions is their basicity rendering sulfate anions to be completely deprotonated at physiological pH whereas phosphate anions exist as a mixture of mono- and diprotonated species under these conditions. The ability of the sulfate-binding protein (SBP) and the phosphate-binding protein (PBP) to differentiate both anions is associated with this property.[3] Whereas SBP contains only hydrogen-bond donors within the active site, PBP contains a single hydrogen-bond acceptor, which can bind to the proton on the HPO_4^{2-} anion. In this way, both proteins maximize the number of possible interactions with the correct substrate while binding of the wrong anion is associated with repulsive interactions rendering binding much weaker.

Sulfate/phosphate selectivity of a number of synthetic anion receptors relies on similar effects.[4,5] Properties of these receptors were often evaluated by determining sulfate

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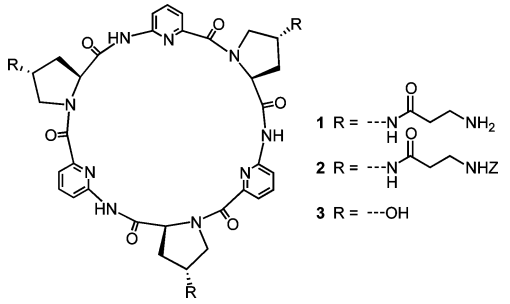
and phosphate affinity independently and the ratio of the corresponding stability constants then provided information about binding preferences. Such studies usually do not yield direct evidence of whether binding of one anion could indeed be achieved in the presence of an excess of the competing anion, however. Here, we introduce cyclopeptide-based receptor **1** whose hydrochloride salt **1**·3HCl possesses appreciable sulfate affinity in water. Importantly, **1**·3HCl binds to sulfate anions even in phosphate buffer, hence in the presence of anions of similar structure but with a different degree of protonation, clearly demonstrating the high sulfate selectivity of this receptor.



Receptor **1** is based on the anion-binding cyclic hexapeptide with L-proline and 6-aminopicolinic acid subunits described by us.[6] The proline subunits contain appended β-alanine residues whose ammonium groups were expected to impart water solubility in addition to directly contributing to anion binding by Coulomb attraction and hydrogen bonding. Indeed, molecular modeling[7] indicated that the triprotonated form of **1** should be well suited to interact with fully deprotonated tetrahedral oxoanions such as sulfate. The graphical abstract shows the calculated structure of the sulfate complex of **1**·3H$^+$. It is evident that three oxygen atoms of the sulfate anion can form hydrogen bonds to the NH groups along the cyclopeptide ring. The same oxygen atoms also form hydrogen bonds to the ammonium groups, each of which forming an additional hydrogen bond to the fourth sulfate oxygen atom. Thus, the substrate is bound by altogether 9 hydrogen bonds not quite reaching the maximum number of 12 hydrogen bonds ideal for coordination of sulfate.[8] A seam of NH···O=C hydrogen bonds between the ammonium groups and the carbonyl groups of the β-alanine residues causes additional stabilization of the complex.

Receptor **1**·3HCl was obtained by reacting a parent cyclopeptide containing (4*R*)-4-aminoproline subunits with Z-protected β-alanine pentafluorophenyl ester followed by hydrogenation of the product in the presence of 3 equiv of HCl (see Supporting Information). The (4*R*)-4-aminoproline subunits in the cyclopeptide originally derive from hydroxyproline. Since natural hydroxyproline also has the *R*-configuration at C-4, substitution of the hydroxyl group by an amino group required two steps, each causing inversion of configuration at C-4.

Qualitative information about the interaction of **1**·3HCl with sulfate anions was obtained from ESI mass spectrometric and ^{1}H NMR spectroscopic studies. For ESI mass spectrometry a 1 mM solution of **1**·3HCl in methanol/water, 1:1 (v/v) adjusted to pH 4.8 by 40 mM acetate buffer was used to ensure complete protonation of the receptor. Mass spectra of this solution showed peaks of monoprotonated **1** $[\mathbf{1} + \mathrm{H}]^+$ and the corresponding sodium adduct $[\mathbf{1} + \mathrm{Na}]^+$ in the positive mode and of the chloride adduct $[\mathbf{1} + \mathrm{Cl}]^-$ in the negative mode as the major species. Addition of 1 equiv of Na_2SO_4 caused the appearance of peaks that could be assigned to cations containing a single sulfate anion ($[\mathbf{1} + 3\mathrm{H} + \mathrm{SO_4}]^+$, $[\mathbf{1} + 2\mathrm{H} + \mathrm{Na} + \mathrm{SO_4}]^+$, $[\mathbf{1} + \mathrm{H} + 2\mathrm{Na} + \mathrm{SO_4}]^+$) (see Supporting Information). No peaks were found, also not in the negative mode, correlating to ions with more than one sulfate anion. Thus, the spectra support the assumption that sulfate ions bind to **1**·3HCl in a 1:1 fashion.

The NMR spectrum of a 1 mM solution of **1**·3HCl in aqueous acetate buffer at pH 4.8 (40 mM CD_3COOD/CD_3COONa in D_2O) and the spectrum obtained after the addition of 2 equiv of Na_2SO_4 are depicted in Figure 1. These spectra show that the presence of the salt causes a downfield shift of the proline H(α) signal. According to previous investigations this shift is a characteristic effect of the interactions of our cyclopeptides with anions.[6]

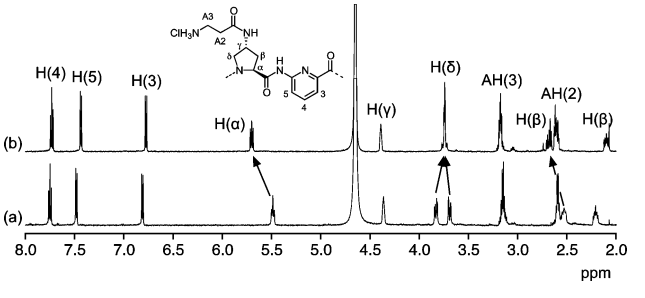


Figure 1. ^{1}H NMR spectrum of a 1 mM solution of **1** in D_2O containing 40 mM CD_3CO_2D/CD_3CO_2Na (pD 4.8) (a) and of a respective solution containing additional 2 equiv of Na_2SO_4 (b).

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(7) For structural optmization DFT/B3LYP/6-31G* calculations were performed using Spartan 10 for Mac (Wavefunction, Inc.). The conformation of the cyclopeptide was based on the crystal structure of the unsubstituted parent compound.[6a]

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Additional changes in the ^{1}H NMR spectrum upon Na_2SO_4 addition involve the signals of the proline H(γ) and H(β) protons as well as those of the aromatic protons accounting for a conformational reorganization of the receptor upon complex formation and/or effects of the anion on the electron density of the corresponding protons in the complex. Changes in the splitting pattern of the β-alanine CH_2 signals were also observed indicating participation of the peripheral substituents of **1** in anion binding. Unfortunately, the lack of meaningful crosspeaks in the NOESY NMR spectrum of **1**·3HCl or its sulfate complex prevented us from obtaining more detailed insights into the complex structure. Nevertheless, the NMR spectroscopic measurements did provide additional evidence for sulfate binding to **1**·3HCl.

Potentiometry in 0.15 M aqueous NaCl solution as the supporting electrolyte was used to determine the protonation constants of the amino groups of **1**. The distribution diagram obtained is shown in Figure 2, and the resulting protonation constants are summarized in Table 1. These constants are in the range typical for primary amines. They decrease with increasing degrees of protonation because of Coulomb repulsion of the protonated ammonium groups, but this effect is smaller than in macrocyclic polyamines,[9] presumably because conformational flexibility of the peripheral substituents in **1** allows the ammonium groups to avoid each other more effectively. Because of the overall basicity of **1**, the fully protonated form dominates at pH 7. Deprotonation only occurs at pH > 7 as evident in Figure 2. At pH > 9 the amide groups in **1** start to deprotonate ($H_{-1}\mathbf{1} + H^+ \leftrightarrows \mathbf{1}$, log K = 9.98(1)) (see Figure 2 and Supporting Information) presumably because they are acidified by the electron-withdrawing nature of the neighboring heterocycles. This assumption is supported by the decrease in intensity of the pyridine band centered at ca. 240 nm in the UV−vis spectra above pH 9.5.

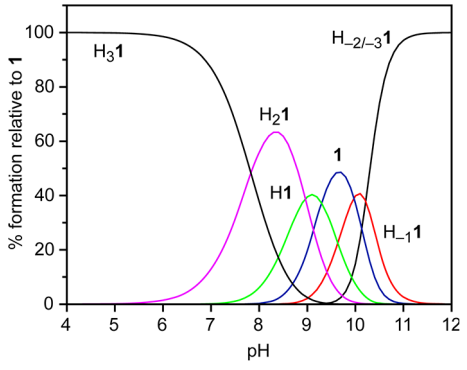

Figure 2. Distribution of the species formed by **1** (c(**1**) = 1 mM) as a function of pH at 298.1 K in 0.15 M aqueous NaCl.

A potentiometric titration in the presence of Na_2SO_4 provided quantitative information about interactions of **1** with sulfate in water. Formation of $[H_p(\mathbf{1})(SO_4)]^{(p-2)}$ species with p = 1, 2, 3 was observed between pH 2.0 and 10.0 with the log K_a values for the reaction $H_p(\mathbf{1})^{p+} + SO_4^{2-} \leftrightarrows [H_p(\mathbf{1})(SO_4)]^{(p-2)}$ increasing with the number of protonated amino groups (log K_a = 3.08(2), 3.44(1), and 4.04(1) for p = 1, 2, and 3, respectively). These values demonstrate that **1** possesses substantial affinity for sulfate even in water and in the presence of an excess of competing chloride anions. The triprotonated $[H_3(\mathbf{1})(SO_4)]^+$ species, in particular, predominates in a wide pH range between 2 and 8 (see Supporting Information).

Further binding studies involved isothermal titration calorimetry. Initial titrations were performed in a mixture of methanol/water, 1:1 (v/v), adjusted to pH 4.8 by the addition of 40 mM acetate buffer. Table 2 shows that with a log K_a of 5.1, **1**·3HCl possesses high sulfate affinity under these conditions. Binding is characterized by favorable enthalpic and entropic contributions of almost equal size. In addition, the stoichiometry factor n denoting the inflection point of the binding isotherm indicates that a 1:1 complex is formed.

Table 1. Protonation Constants (log K) of **1** Determined Potentiometrically in 0.15 M Aqueous NaCl at 298.1 K

reaction	log K
$H_{-1}\mathbf{1}^- + H^+ \leftrightarrows \mathbf{1}$	9.98(2)[a]
$\mathbf{1} + H^+ \leftrightarrows H\mathbf{1}^+$	9.29(2)
$H\mathbf{1}^+ + H^+ \leftrightarrows H_2\mathbf{1}^{2+}$	8.95(1)
$H_2\mathbf{1}^{2+} + H^+ \leftrightarrows H_3\mathbf{1}^{3+}$	7.84(1)

[a] Values in parentheses are standard deviations in the last significant figure.

Table 2. Stoichiometry Factor n, Association Constants log K_a, Gibbs Energies ΔG, Enthalpies ΔH, and Entropies $T\Delta S$ of Binding of Na_2SO_4 and NaOTs to Cyclopeptides **1, 2**, and **3** at 298 K[a]

		n	log K_a	ΔH	$T\Delta S$
Na_2SO_4	**1**	0.85[b]	5.10 (0.04)	−13.1 (0.4)	16.0 (0.6)
	2	1[c]	3.57 (0.06)	−13.8 (0.7)	6.6 (1.1)
	3	1[c]	2.36 (0.01)	−10.6 (0.3)	2.9 (0.3)
NaOTs	**1**	1[c]	2.80 (0.07)	−13.8 (0.6)	2.2 (0.9)
	2	1[c]	3.16 (0.03)	−21.0 (0.9)	−2.9 (1.0)

[a] All measurements were performed at least three times in methanol/water, 1:1 (v/v) containing 40 mM acetate buffer (pH 4.8), standard deviations are specified in parentheses, energies are given in kJ·mol^{-1}. [b] Stoichiometry factor n fitted. [c] Stoichiometry factor n fixed at 1.

For comparison, titrations were also performed by using sodium tosylate (NaOTs) as the substrate or cyclopeptides **2** and **3** as receptors. Interactions turned out to be considerably weaker in all of these titrations, and a simultaneous fitting of K_a, ΔH, and n was associated with larger errors. Therefore, these binding isotherms were fitted under the reasonable assumption of 1:1 complex formation with n fixed at 1.[10]

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Table 2 shows that cyclopeptide **3** lacking the β-alanine residues forms a significantly less stable complex with sulfate anions than **1**, demonstrating that the anion affinity of **1** strongly benefits from electrostatic interactions. Moreover, sulfate binding of **1** is considerably stronger than tosylate binding, which lends support to the calculated structure of the sulfate complex of **1** involving multiple interactions between host and guest. A similar structure is not possible for tosylate. To exclude that the higher stability of the sulfate complex is only due to the higher charge of sulfate anions, titrations were also performed with the neutral cyclopeptide **2** containing protected amino groups in the side chains. The results show that **2** has almost the same affinity for sulfate and tosylate anions, which indicates that electostatic interactions do not determine complex stability alone. In addition, the sulfate complex of **2** is more than 1 order of magnitude less stable than that of **1** suggesting that the high stability of the sulfate complex of **1** is likely due to a special complexation mode, possibly similar to the one shown in the graphical abstract. Comparison of the results in Table 2 furthermore shows that the high stability of the sulfate complex of **1** has entropic reasons since sulfate and tosylate complexation of **1** is associated with almost the same enthalpy change.

We next turned our attention to ITC titrations in water, the results of which are summarized in Table 3.

Table 3. Stoichiometry Factor n, Association Constants log K_a, Gibbs Energies ΔG, Enthalpies ΔH, and Entropies $T\Delta S$ of Binding of Na_2SO_4 to Receptor **1** at 298 K under Different Conditions[a]

	pH	n	log K_a	ΔH	$T\Delta S$
AcOH/AcONa[b]	4.8	0.88[d]	4.20 (0.01)	−13.5 (0.1)	10.5 (0.1)
NaH_2PO_4/Na_2HPO_4[b]	7.0	1[e]	2.44 (0.01)	−3.4 (0.1)	10.5 (0.1)
NaH_2PO_4[b]	4.6	1[e]	3.62 (0.15)	−5.2 (0.2)	15.5 (1.1)
H_3PO_4/NaH_2PO_4[b]	2.3	1[e]	3.88 (0.13)	−7.3 (0.7)	14.8 (1.4)
aqueous NaCl[c]		1[e]	2.87 (0.01)	−4.3 (0.1)	12.1 (0.1)

[a] All measurements were performed at least three times, standard deviations are specified in parentheses, and energies are given in $kJ \cdot mol^{-1}$. [b] Buffer concentrations are 40 mM in water. [c] Salt concentration = 0.15 M. [d] Stoichiometry factor n fitted. [e] Stoichiometry factor n fixed at 1.

When the solvent is changed from methanol/water, 1:1 (v/v), to water and the buffer concentration and the pH are kept constant, the sulfate affinity of **1** decreases by 1 order of magnitude but still has an appreciable log K_a value of 4.2. More importantly, the acetate buffer can even be replaced by a phosphate buffer without fully compromising anion binding.[11] Sulfate affinity under these conditions increases when lowering the pH, but even at pH 2.3 it does not reach the value obtained in acetate buffer at pH 4.8. This result shows that phosphate anions, as expected for tetrahedral oxoanions, compete more strongly for binding to the cyclopeptide than acetate anions, whose geometry does not match the binding site of the host very well. Nevertheless, phosphate anions are inferior guests for **1** with respect to sulfate anions because they are partially protonated in the pH range used so that repulsive interactions are unavoidable with a host containing only hydrogen bond donors as binding sites. As a consequence, binding of **1** to phosphate anions is weaker. The fact that sulfate anions are bound more strongly at lower pH at which the degree of protonation of phosphate ions increases is in line with this interpretation. It should also be noted that the reduction in complex stability when changing the acetate for a phosphate buffer is mainly due to a reduction in the enthalpic contribution to binding. The stability constant of the sulfate complex determined by ITC in 0.15 M aqueous NaCl is lower but overall similar to the values determined potentiometrically under comparable conditions, lending support to the reliability of the data.

In conclusion, our results demonstrate the high affinity and selectivity of **1** for sulfate anions in aqueous solution even in the presence of an excess of competing anions such as AcO^-, Cl^-, and HPO_4^{2-} or $H_2PO_4^-$.[11] This property is presumably caused by a combination of electrostatic interactions with the ability of **1** to wrap around a tetrahedral oxoanion with the hydrogen bond donor sites converging toward the guest. Although our results do not provide a quantitative estimate for the sulfate/phosphate selectivity of **1**, selectivity is obviously sufficiently high to allow for sulfate binding even in an aqueous phosphate buffer. This cyclopeptide therefore represents a promising lead structure for the development of sulfate sensors in water. Future studies will also address the binding of other tetrahedral oxoanions such as selenate or perrhenate to correlate binding strength with anion properties.

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Supporting Information Available. Synthesis and spectroscopic characterization of **1**. Experimental details and results of the binding studies (ESI-mass spectra, potentiometric measurements, ITC titrations). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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