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Tetrahedron Letters

Tetrahedron Letters 45 (2004) 4273–4276

Thiourea–isothiouronium conjugate for strong and selective binding of very hydrophilic $H_2PO_4^-$ anion at the 1,2-dichloroethane–water interface

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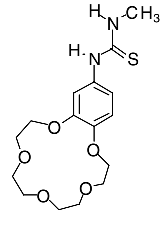
Received 4 March 2004; revised 30 March 2004; accepted 5 April 2004

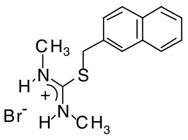
Abstract—Potential use of a surfactant-like receptor is demonstrated at the 1,2-dichloroethane–water interface for strong and selective binding of $H_2PO_4^-$ over Br^- and Cl^-. The analysis by interfacial tensiometry reveals that the interfacial adsorption of a thiourea–isothiouronium conjugate, BT-C_1, is significantly stabilized by the binding of $H_2PO_4^-$ with the adsorption constant of $1.7 \times 10^5\,M^{-1}$ while the interfacial adsorptivity of this receptor is relatively moderate for Br^- ($0.81 \times 10^5\,M^{-1}$) and Cl^- ($0.63 \times 10^5\,M^{-1}$). Such complexation-induced interfacial adsorption behaviors of BT-C_1 are discussed as a basis for the development of receptor-based chemical sensors for phosphate anions.

The development of phosphate-selective receptors is of ongoing interest due to their potential applications in biological researches.[1,2] Whereas selective binding and sensing of phosphates and their derivatives have been successfully demonstrated by sophisticated receptors in bulk solutions, use of such receptor molecules in chemical sensors does not always result in phosphate selectivity.[3] Indeed, it is still highly challenging to achieve useful selectivity for phosphates over hydrophobic anions by receptor-based chemical sensors such as ion-selective electrodes (ISEs).[4–6] This is due to the high hydrophilicity of phosphate anions, which leads to the difficult problem of compensating for the large differences in Gibbs free energies of transfer between phosphates and interfering anions by selective complexation. Therefore, despite the originally designed functions of receptors in bulk media, the anion selectivity of two-phase distribution systems is significantly affected by the order of the free energy of hydration ($-\Delta G_h$) of anions: $H_2PO_4^- > Cl^- > Br^-$.

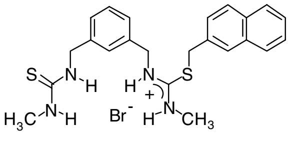
To achieve phosphate selectivity in two-phase distribution systems, our particular interest has been directed at complexation-induced interfacial adsorption behaviors by surfactant-like receptors at the oil–water interface.[7,8] We have recently reported that,[7] at the 1,2-dichloroethane (DCE)–water interface, thiourea-functionalized benzo-15-crown-5 (TU-B15C5), present in the DCE phase, does selectively recognize $H_2PO_4^-$ over Cl^-, Br^-, and CH_3COO^- in the aqueous phase, and this is accompanied by the interfacial adsorption of the



TU-B15C5



MT-C_1



BT-C_1

Keywords: Thiourea; Isothiouronium; Receptor; Anion; Phosphate; Recognition; Adsorption; Interface.

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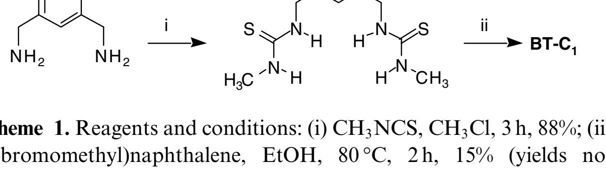
0040-4039/$ - see front matter
doi:10.1016/j.tetlet.2004.04.001

complex. Our study suggests that the solvating environment at the interface[9,10] is inherently suitable for the recognition of hydrophilic substrates: in contrast to bulk two-phase distribution systems, it is likely that complete dehydration of $H_2PO_4^-$ is not required for the interfacial binding event, which would make it possible to selectively recognize $H_2PO_4^-$ over hydrophobic anions.[11] It is therefore expected that use of surfactant-like receptors offers a potential basis for the design of receptor-based chemical sensors for phosphate anions. In our previous system based on TU-B15C5,[7] however, the complexation-induced interfacial adsorptivity was only moderate: the adsorption constant of TU-B15C5 was $0.15\times10^5\,M^{-1}$ in the presence of 0.1 M $H_2PO_4^-$ in the aqueous phase.

We now show that strong and selective binding of $H_2PO_4^-$ can be achieved by a thiourea–isothiouronium conjugate, BT-C_1, at the DCE–water interface. It is characteristic of BT-C_1 to have two types of hydrogen bond forming sites, that is, thiourea[12,13] and isothiouronium[14,15] groups, both of which are suitable for phosphate binding, and these two binding sites are linked by a *m*-xylene spacer[12a] so as to bind to target phosphates through the formation of multiple hydrogen bonds. The analysis by interfacial tensiometry reveals that the adsorption constant of this receptor reaches $(1.7\pm0.06)\times10^5\,M^{-1}$ upon complexation with $H_2PO_4^-$ at the interface. Comparison with interfacial binding behaviors of mono-thiouronium MT-C_1 shows it is most likely that both thiourea- and isothiouronium-binding sites are involved in the anion binding, by which a highly stable complex forms between BT-C_1 and $H_2PO_4^-$ at the DCE–water interface.

The synthesis of MT-C_1 was described previously.[15a] BT-C_1 was synthesized in two steps starting from 1,3-bis-(aminomethyl)benzene. As is shown in Scheme 1, the reaction with methyl isothiocyanate gave α,α′-bis-(*N*′-methylthioureylene)-*m*-xylene, which was then reacted with 2-(bromomethyl)naphthalene. Purification by silica gel column chromatography (CH_3Cl–CH_3OH, 8:1) gave the product as a bromide salt.[16]

Firstly, we examined the effect of $H_2PO_4^-$ anions on the time course of the interfacial tension (dynamic interfacial tension, $\gamma(t)$) at the DCE–water interface.[17] As is shown in Figure 1A, a steep decrease in the interfacial tension is observed irrespective of the absence or presence of target $H_2PO_4^-$, and the dynamic interfacial tension reaches equilibrium within a short time range. This indicates that BT-C_1 adsorbs at the interface even in the absence of $H_2PO_4^-$. However, compared to results in the absence of $H_2PO_4^-$ (curve a), the decrease in $\gamma(t)$ is significantly larger in the presence of $H_2PO_4^-$ (curve b). Obviously, the interfacial adsorptivity of BT-C_1 is enhanced in the presence of $H_2PO_4^-$, indicating the complex formation between BT-C_1 and $H_2PO_4^-$ does take place at the interface, which is accompanied by the interfacial adsorption of the complex. When the adsorption kinetics is controlled by the diffusion of BT-C_1 from the bulk DCE phase to the interface, $\gamma(t)$ is given by the following equation:[18,19]



Scheme 1. Reagents and conditions: (i) CH_3NCS, CH_3Cl, 3 h, 88%; (ii) 2-(bromomethyl)naphthalene, EtOH, 80 °C, 2 h, 15% (yields not optimized).

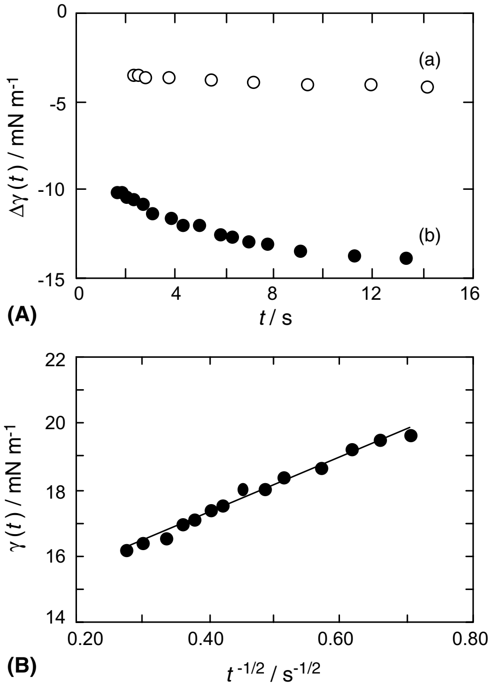


Figure 1. (A) Dynamic interfacial tension at the 1,2-dichloroethane (DCE)–water interface. Aqueous phase: (a) pure water or (b) 0.1 M $H_2PO_4^-$ (as Na^+ salt); DCE phase: 1.0×10^{-4} M of BT-C_1. (B) Relationship between $\gamma(t)$ and $t^{-1/2}$ analyzed for curve (b) in Figure 1A.

$$\gamma(t) = \gamma_e + (RT\Gamma_e^2/[\mathrm{BT}-\mathrm{C}_1]) \times (\pi/4Dt)^{1/2} \quad (1)$$

where γ_e is the equilibrium interfacial tension ($mN\,m^{-1}$), Γ_e is the equilibrium interfacial concentration of BT-C_1 ($mol\,m^{-2}$), and D is the diffusion coefficient of BT-C_1 in the DCE phase ($m^2\,s^{-1}$). Since the plot of $\gamma(t)$ against $t^{-1/2}$ gives a linear relationship (Fig. 1B), the diffusion of BT-C_1 from the bulk to the interface is characterized as a rate-determining process for the interfacial adsorption and binding event.

The thermodynamics of adsorption behaviors of BT-C_1 was then analyzed on the basis of Gibbs adsorption isotherms. For comparison, the adsorption behaviors of MT-C_1 were also examined. Figure 2 shows a typical Gibbs plot for BT-C_1 (a) in the absence or (b) presence of 0.1 M $H_2PO_4^-$, where the change in the equilibrium interfacial tension $\Delta\gamma$ is plotted as a function of the receptor concentration in the organic phase. The adsorption parameters can be evaluated by nonlinear fitting with the following Szyszkowski equation:[20]

$$\Delta\gamma = \gamma_e - \gamma_0 = -\Gamma_{\mathrm{satd}}RT\ln(1 + K_{\mathrm{ad}}[\mathrm{receptor}]) \quad (2)$$

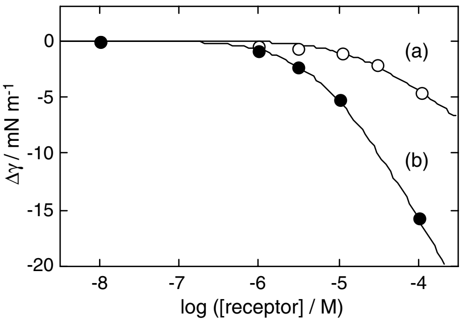


Figure 2. Gibbs plot. Aqueous phase: (a) pure water or (b) 0.1 M $H_2PO_4^-$ (as Na^+ salt); DCE phase: 1.0×10^{-8}–1.0×10^{-4} M of BT-C_1.

where γ_0 is the equilibrium interfacial tension without receptors ($mN\,m^{-1}$), Γ_{satd} is the saturated interfacial concentration of receptor ($mol\,m^{-2}$), and K_{ad} is the adsorption constant (M^{-1}). The effect of several anions on K_{ad} and Γ_{satd} of both receptors is summarized in Table 1.[21]

As can be seen from Table 1, BT-C_1 shows clear selectivity for $H_2PO_4^-$ over Br^- and Cl^- at the DCE–water interface. While the adsorption constants K_{ad} ($10^5\,M^{-1}$) for Br^- (0.81 ± 0.23) and Cl^- (0.63 ± 0.22) are almost equal within the experimental errors, the K_{ad} for $H_2PO_4^-$ (1.7 ± 0.06) is significantly larger than that in the absence of these anions (0.54 ± 0.24). By contrast, such a phosphate selectivity is not obtained by mono-isothiouronium MT-C_1, where the strongest adsorption is observed not for $H_2PO_4^-$ (0.30 ± 0.07), but for Br^- (0.56 ± 0.11). It is therefore most likely that BT-C_1 binds to $H_2PO_4^-$ cooperatively by two binding sites (Fig. 3), as has been suggested for the $H_2PO_4^-$ binding by structurally similar bis-(thio)urea receptors in bulk DMSO-d_6.[12a]

Table 1. Effect of anions on the adsorption parameters of MT-C_1 and BT-C_1 at the 1,2-dichloroethane–water interface

	Anion[a]	K_{ad} ($10^5\,M^{-1}$)[b]	Γ_{satd} ($10^{-6}\,mol\,m^{-2}$)[c]
MT-C_1	No guest	0.056 ± 0.033	0.78 ± 0.28
	Br^-	0.56 ± 0.11	0.62 ± 0.07
	Cl^-	0.25 ± 0.18	0.82 ± 0.37
	$H_2PO_4^-$	0.30 ± 0.07	0.70 ± 0.06
BT-C_1	No guest	0.54 ± 0.24	0.96 ± 0.22
	Br^-	0.81 ± 0.23	3.0 ± 0.45
	Cl^-	0.63 ± 0.22	4.0 ± 0.72
	$H_2PO_4^-$	1.7 ± 0.06	2.2 ± 0.03

[a] 0.1 M solution of Na^+ salts.

[b] The adsorption constant.

[c] The saturated interfacial concentration.

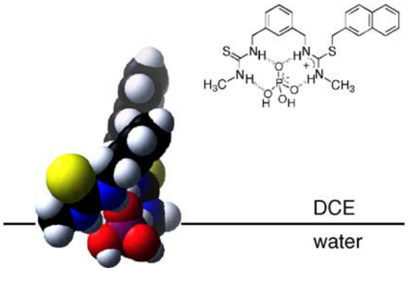


Figure 3. A possible binding structure of the BT-C_1/$H_2PO_4^-$ complex.

Table 1 also shows that anion binding causes a significant change in the saturated interfacial concentration (Γ_{satd}) of BT-C_1. In the absence of target anions, Γ_{satd} of BT-C_1 is $(0.96\pm0.22)\times10^{-6}\,mol\,m^{-2}$, and increases to $(2.2\pm0.03)\times10^{-6}\,mol\,m^{-2}$ upon complexation with $H_2PO_4^-$ anions.[22] This corresponds to the change in the apparent molecular occupied area of BT-C_1 from 180 to 76 Å². The BT-C_1/$H_2PO_4^-$ complex is therefore able to be adsorbed at the interface in a highly packed fashion since the molecular area of the complex is estimated to be ca. 60–140 Å² from the CPK model by assuming that this receptor adsorbs vertically at the interface (Fig. 3).

In summary, we have demonstrated complexation-induced interfacial adsorption of BT-C_1 at the DCE–water interface. This surfactant-like receptor, BT-C_1, showed the strongest adsorptivity for $H_2PO_4^-$ over Br^- and Cl^-, and the cooperative binding by two types of hydrogen bond forming sites seemed to be responsible for strong and selective binding of $H_2PO_4^-$. From the results presented here and in our previous work, it is highly likely that the solvating environment at the interface is suitable for the recognition of hydrophilic anions, and the use of well-designed receptors at the interface is expected to provide a potential basis for the development of phosphate-selective chemical sensors.

Acknowledgements

We thank Drs. Hinako Ando and Kazuo Sasaki, Instrumental Analysis Center for Chemistry, Faculty of Science, Tohoku University, for microanalyses and ^1H NMR measurements. This work was partially supported by Grants-in-Aid for Scientific Research (A), No 14204074, for Scientific Research on Priority Research Areas (B), No 13129201, and for the COE Project, Giant Molecules and Complex Systems, 2003, from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. Partial support by RFTF from the Japan Society for Promotion of Science (JSPS) is also acknowledged.

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17. We measured the interfacial tension at the DCE–water interface by the dynamic drop volume method.[18,19] For this, a glass capillary (0.705 cm o.d.) was fixed in a glass cell filled with a DCE solution of receptors. An aqueous solution containing anions (0.1 M, as Na$^+$ salts) was sent upward into the DCE solution as a series of droplets by injecting it through the glass capillary using a syringe pump. The dynamic interfacial tension $\gamma(t)$ was calculated from the volume of droplets as a function of the introduction time, and then the equilibrium interfacial tension γ_e was obtained by plotting $\gamma(t)$ against $t^{-1/2}$. All experiments were carried out at 298 K.
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21. Due to the difference in ionic strength between pure water and a 0.1 M aqueous electrolyte solution, the adsorption constants of receptor molecules in the absence of target anions are calculated to be relatively large as compared to those in the presence of target anions.
22. The large increase in Γ_{satd} observed for Br$^-$ and Cl$^-$ may be ascribed to the ability of these anions to neutralize the positively charged species, that is, the isothiouronium binding site of BT-C$_1$, which would reduce the charge repulsion between receptors adsorbed at the interface.