Selective recognition of dihydrogen phosphate by receptors bearing pyridyl moieties as hydrogen bond acceptors†

Shin-ichi Kondo,* Yuichi Hiraoka, Namiko Kurumatani and Yumihiko Yano

Received (in Cambridge, UK) 12th November 2004, Accepted 25th January 2005
First published as an Advance Article on the web 2nd February 2005
DOI: 10.1039/b417304j

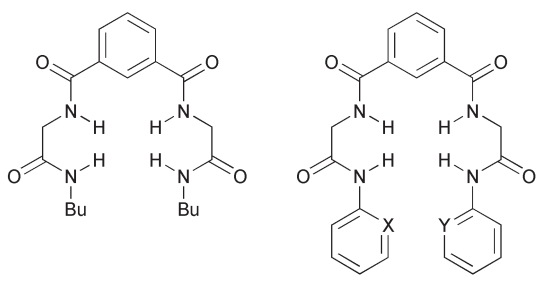
Dihydrogen phosphate anion is selectively recognized by amide-based receptors bearing pyridyl moieties as hydrogen bond acceptors in 0.5% DMSO–acetonitrile.

Artificial anion receptors have attracted considerable attention due to their medicinal and environmental potential.[1] Phosphate anions are very important anionic species in living organisms. Naturally occurring phosphate-binding protein (PBP) and sulfate-binding protein selectively and strongly bind hydrogen phosphate and sulfate anions, respectively.[2] Hydrogen bond of backbone amide NH groups and the side chains of amino acid residues to the guest anionic species are important for the binding sites of these proteins. One remarkable difference between these two proteins is the existence of a hydrogen bond acceptor in PBP, namely, the carboxylate of the aspartate residue plays a crucial role as a hydrogen bond *acceptor* of the phosphate OH to discriminate among analogous tetrahedral anionic guests such as sulfate anions. Recently, phosphate ion selective receptors have been reported.[3–5] In many cases, tripodal receptors were used to complex with a tetrahedral phosphate anion to create a C_{3v} symmetric cavity.[4] However, few studies have reported receptors bearing a hydrogen bond acceptor for discriminating between (di)hydrogen phosphate and other guest anions. Wu and co-workers reported that receptors based on a tris(aminoethyl)amine (tren) skeleton bearing three thiourea or urea groups selectively bind dihydrogen phosphate anion in DMF, and proposed hydrogen bonding between the phosphate OH and the nitrogen of the tertiary amine of the tren skeleton.[5] Amidopyridine derivatives associate with carboxylic acid *via* hydrogen bonding and a number of artificial receptors for mono- and dicarboxylic acids have been reported.[6] By introducing amidopyridine moieties into an anion receptor, selective recognition of dihydrogen phosphate should be possible by adding a hydrogen bond acceptor for the OH group in the target anion. Here, we demonstrate the selective recognition of dihydrogen phosphate anion by tetramide receptors bearing pyridyl moieties by combining hydrogen bond donor and acceptor moieties in a polar aprotic solvent.

Receptor molecules **1** and **2** consist of three parts, *i.e.*, isophthalamide, α-amino acid spacer, and N-terminal functionalities, as shown in Chart 1. All four NH groups of the amide form a complex with Y-shaped anions such as acetate and phosphate. Functionalization can be achieved easily by introducing N-terminal groups and/or replacement of α-amino acid moieties *via* well-established peptide synthetic methods. The pyridyl groups of **2c** should form additional hydrogen bonds with the hydroxy groups in dihydrogen phosphate anions.

Methods of preparing receptors **1** and **2** are illustrated in Fig. 1. Glycine protected with a benzyloxycarbonyl group was condensed with 1-butylamine, aniline, or 2-aminopyridine with DCC in the presence of HOBt in *N*,*N*-dimethylformamide. Deprotection of the benzyloxycarbonyl group was accomplished by hydrogenation in the presence of Pd/C in MeOH or EtOH, followed by reaction between isophthalic acid and DCC to give the receptors **1** and **2**, respectively. Half amide **5** was prepared from **3** with 1 equiv. of isophthalic acid with DCC/HOBt and the product converted to asymmetric tetraamide **2b** by condensation with **4**[7] in the presence of DCC. Products were characterized by ^{1}H NMR, ESI-MS, and elemental analysis.

In the ^{1}H NMR titration experiment, the addition of tetra(*n*-butyl)ammonium acetate to a solution of **1** in DMSO-d_6 resulted in the downfield shift of both amide NH groups (Fig. S1, see ESI†), suggesting that the amide NH moieties act as hydrogen bond donors. The association constant of **1** with AcO^- was determined to be 91 dm^3 mol^{-1}. Fig. 2 presents the changes in the UV-vis spectra of **2c** upon addition of 4 equiv. of oxoanions, such as AcO^-, $H_2PO_4^-$, and HSO_4^- in less polar aprotic solvent, 0.5% DMSO–MeCN (v/v). In the absence of anions, **2c** showed an absorption maximum at 274.5 nm. Upon the addition of AcO^- and HSO_4^-, small bathochromic shifts were observed. However, upon addition of $H_2PO_4^-$, a large bathochromic shift ($\Delta\lambda_{max}$ = 15.5 nm) was observed. The receptor **2a** showed a small bathochromic shift upon the addition of AcO^-, $H_2PO_4^-$, and HSO_4^-. These results indicate the receptor **2c** can discriminate spectrophotometrically between $H_2PO_4^-$ and the structurally



1

2a : X = Y = CH
2b : X = N, Y = CH
2c : X = Y = N

Chart 1

† Electronic supplementary information (ESI) available: Experimental procedure for the preparation of receptors **1** and **2**, ^{1}H NMR spectral changes of **1** upon the addition of AcO^-, ESI-MS of complexes, and UV-vis spectral changes of **2c** with $H_2PO_4^-$. See http://www.rsc.org/suppdata/cc/b4/b417304j/

*kondo@chem.gunma-u.ac.jp

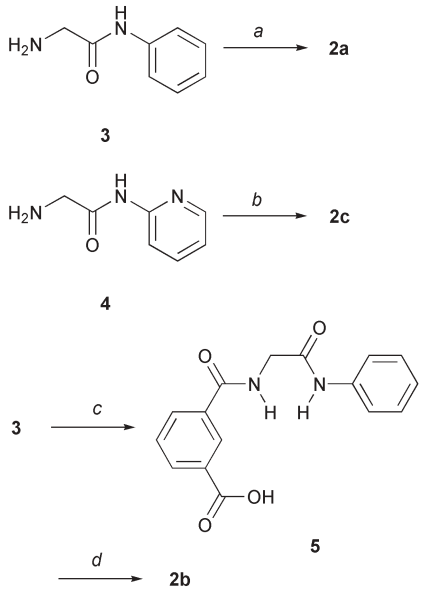


Fig. 1 Synthesis of **1** and **2**. Reagents and Conditions: (a) isophthalic acid (0.43 equiv.), DCC, HOBt, DMF, 78%; (b) isophthalic acid (0.44 equiv.), DCC, HOBt, DMF, 61%; (c) isophthalic acid (1 equiv.), DCC, HOBt, DMF, 41%; (d) **4**, DCC, HOBt, DMF, 62%.

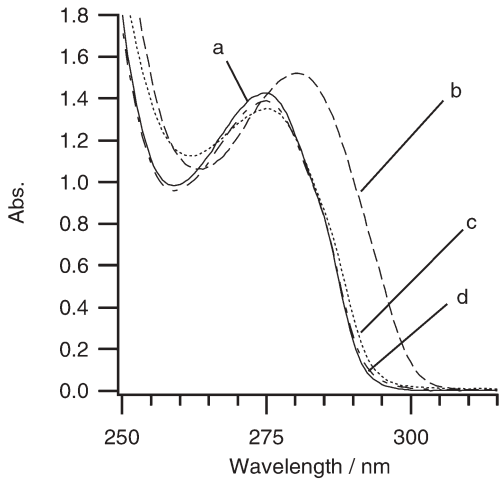


Fig. 2 UV-vis spectra of **2c** (1.0×10^{-4} mol dm^{-3}) in the absence of anions (a) and in the presence of 4 equiv. of $H_2PO_4^-$ (b), AcO^- (c), and HSO_4^- (d) in 0.5% DMSO–MeCN (v/v) at 298 K.

related AcO^- and HSO_4^- anions. It is noteworthy that the addition of hydrogen sulfate anion caused only small spectral changes, as shown in Fig. 2. The possibility that the large spectral changes occurring upon addition of $H_2PO_4^-$ arise from proton transfer from the hydroxy group in $H_2PO_4^-$ to the pyridyl group because $H_2PO_4^-$ possesses lower acidity than does HSO_4^- was rejected. In the UV-vis titration of **2** with guest anions in 0.5% DMSO–MeCN (v/v), spectral changes occurring at isosbestic points clearly show 1 : 1 complex formation. ESI-MS results also support the formation of a complex. Peaks corresponding to a 1 : 1 complex agreed well with the isotope patterns; no higher order complexes were observed. (Fig. S2, see ESI†) The association constants were calculated by non-linear curve fitting of UV-vis titration data with a 1 : 1 complexation model. (Fig. S4, see ESI†) The association constants of **1** and **2** for AcO^-, $H_2PO_4^-$, $(EtO)_2PO_2^-$, and HSO_4^- are summarized in Table 1.

The association constants of **2a** for AcO^- and $H_2PO_4^-$ were slightly larger than those of **1**, which is attributed to the relatively strong hydrogen bond by the amide NH of **2a** rather than the amide of **1**, due to the strong acidity of an acylanilide NH compared to that of an alkylamide. However, the selectivities of **1** and **2a** for $H_2PO_4^-$ [$K_{11}(H_2PO_4^-)/K_{11}(AcO^-)$] were similar, as shown in Table 1. The association constant of **2c** for AcO^- was the same order of magnitude as that for **2a** within experimental error. Interestingly, the association constant of **2c** for $H_2PO_4^-$ is significantly larger than that of **2a**. The association constant of **2c** for $H_2PO_4^-$ is too large to determine accurately by UV-vis spectroscopic titration; phosphate selectivity of **2c** was >59.9. The association constants of **2c** for $H_2PO_4^-$ and AcO^- in more polar aprotic solvent, 10% DMSO–MeCN (v/v) were determined to be 1.20×10^5 and 1.41×10^3 dm^3 mol^{-1}, respectively ($K_{11}(H_2PO_4^-)/K_{11}(AcO^-) = 85.1$). These results indicate that the receptor bearing pyridyl moiety **2c** can discriminate between anionic species, *i.e.*, acetate and dihydrogen phosphate. The association constants of **2a** and **2c** for diethyl phosphate monoanion also were the same order of magnitude‡, indicating that the hydroxy group in $H_2PO_4^-$ plays a critical role in discrimination.

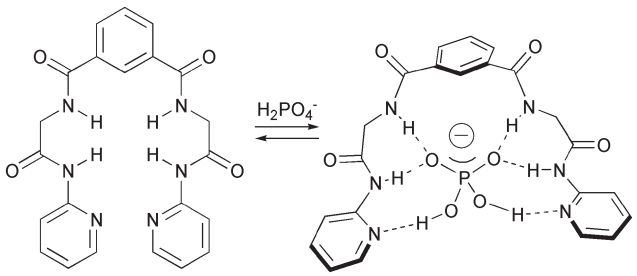
The properties of **2b** bearing pyridyl and phenyl groups at the N-terminal indicates whether one or two pyridyl groups of **2c** are necessary to recognize $H_2PO_4^-$. As shown in Table 1, the association constants of **2b** for anions are between those of **2a** and **2c**, indicating that two pyridyl groups of **2c** and two hydroxy groups of $H_2PO_4^-$ form hydrogen bond pairs. The proposed structure of the complex formed by **2c** and $H_2PO_4^-$ is shown in Scheme 1. Four amide NH groups act as hydrogen bond donors to recognize anionic oxygen atoms of $H_2PO_4^-$, and two pyridyl groups act as hydrogen bond acceptors to recognize the hydroxy groups in $H_2PO_4^-$, as observed in PBP.

In conclusion, we demonstrate a tetramide-based receptor bearing pyridyl moieties, **2c**, that possesses remarkable selectivity for $H_2PO_4^-$ in 0.5% DMSO–MeCN (v/v). Selectivity is achieved

Table 1 The association constants of receptors **1** and **2** with anions

	K_{11}/dm^3 mol^{-1}[a]				
Receptor	AcO^-	$H_2PO_4^-$	$(EtO)_2PO_2^-$	HSO_4^-	Phosphate selectivity[b]
1	8.75×10^3	9.56×10^3		<100	1.1
2a	2.22×10^4	2.58×10^4	6.84×10^4	<100	1.1
2b	1.83×10^4	5.64×10^5	4.12×10^4	<100	30.8
2c	1.67×10^4	$>10^6$	1.71×10^4	6.1×10^2	>59.9

[a] Tetra(*n*-butyl)ammonium ion was used for counter ion. Determined by UV-vis spectroscopy in 0.5% DMSO–MeCN (v/v) at 298 K. [Receptor] = 1.0×10^{-4} mol dm^{-3}. The errors in the association constants were less than 10%. [b] $K_{11}(H_2PO_4^-)/K_{11}(AcO^-)$.



Scheme 1

by the hydrogen bond acceptor of the pyridyl group acting as an active site of the phosphate binding protein. We believe that the results presented will be useful for the design of more sophisticated receptors for phosphate derivatives, such as co-enzymes.

Shin-ichi Kondo,* Yuichi Hiraoka, Namiko Kurumatani and Yumihiko Yano

Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan. E-mail: kondo@chem.gunma-u.ac.jp; Fax: +81 277 30 1236; Tel: +81 277 30 1236

Notes and references

‡ As the number of pyridyl groups increased (**2a–c**), the association constants for $(EtO)_2PO_2^-$ decreased slightly, possibly due to the additional CH–π interaction of ethyl groups and phenyl groups. This phenomenon will be discussed in detail elsewhere.

1 For recent reviews: (*a*) A. Bianchi, K. Bowman-James and E. Garcia-Espana, *Supramolecular Chemistry of Anions*; Wiley-VCH, New York, 1997; (*b*) For recent reviews: J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 89; (*c*) F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609; (*d*) M. M. G. Antonisse and D. N. Reinhoudt, *Chem. Commun.*, 1998, 443; (*e*) P. A. Gale, *Coord. Chem. Rev.*, 2000, **199**, 181; (*f*) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486.

2 (*a*) H. Luecke and F. A. Quiocho, *Nature*, 1990, **347**, 402; (*b*) J. J. He and F. A. Quiocho, *Science*, 1991, **251**, 1497; (*c*) J. W. Pflugrath and F. A. Quiocho, *Nature*, 1985, **314**, 257.

3 (*a*) D. M. Rudkevich, W. Verboom and D. N. Reinhoudt, *J. Org. Chem.*, 1994, **59**, 3683; (*b*) D. M. Rudkevich, W. Verboom, Z. Brzozka, M. J. Palys, W. P. R. V. Stauthamer, G. J. van Hummel, S. M. Franken, S. Harkema, J. F. J. Engbersen and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1994, **116**, 4341; (*c*) P. Bühlmann, S. Nishizawa, K. P. Xiao and Y. Umezawa, *Tetrahedron*, 1997, **53**, 1647; (*d*) Y. Tobe, S. Sasaki, M. Mizuno and K. Naemura, *Chem. Lett.*, 1998, 835; (*e*) P. Anzenbacher, Jr., K. Jursikova and J. L. Sessler, *J. Am. Chem. Soc.*, 2000, **122**, 9350; (*f*) D. H. Lee, H. Y. Lee, K. H. Lee and J.-I. Hong, *Chem. Commun.*, 2001, 1188; (*g*) M. S. Han and D. H. Kim, *Angew. Chem., Int. Ed.*, 2002, **41**, 3809; (*h*) M. A. Hossain, S. O. Kang, D. Powell and K. Bowman-James, *Inorg. Chem.*, 2003, **42**, 1397; (*i*) L.-J. Kuo, J.-H. Liao, C.-T. Chen, C.-H. Huang, C.-S. Chen and J.-M. Fang, *Org. Lett.*, 2003, **5**, 1821; (*j*) H. Tong, G. Zhou, L. Wang, X. Jing, F. Wang and J. Zhang, *Tetrahedron Lett.*, 2003, **44**, 131; (*k*) J. Yoon, S. K. Kim, N. J. Singh, J. W. Lee, Y. J. Yang, K. Chellappan and K. S. Kim, *J. Org. Chem.*, 2004, **69**, 581; (*l*) M. J. Chmielewski, M. Charon and J. Jurczak, *Org. Lett.*, 2004, **6**, 3501.

4 (*a*) P. D. Beer, C. A. P. Dickson, N. Fletcher, A. J. Goulden, A. Grieve, J. Hodacova and T. Wear, *J. Chem. Soc., Chem. Commun.*, 1993, 828; (*b*) C. Raposo, M. Almaraz, M. Martin, V. Weinrich, M. a. L. Mussons, V. Alcazar, M. C. Caballero and J. R. Moran, *Chem. Lett.*, 1995, 759; (*c*) S. Sasaki, D. Citterio, S. Ozawa and K. Suzuki, *J. Chem. Soc., Perkin Trans. 2*, 2001, 2309; (*d*) S. L. Tobey and E. V. Anslyn, *Org. Lett.*, 2003, **5**, 2029; (*e*) H. R. Seong, D.-S. Kim, S.-G. Kim, H.-J. Choi and K. H. Ahn, *Tetrahedron Lett.*, 2004, **45**, 723.

5 (*a*) H. Xie, S. Yi and S. Wu, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2751; (*b*) H. Xie, S. Yi, X. Yang and S. Wu, *New J. Chem.*, 1999, **23**, 1105.

6 For recent reviews: (*a*) R. J. Fitzmaurice, G. M. Kyne, D. Douheret and J. D. Kilburn, *J. Chem. Soc., Perkin Trans. 1*, 2002, 841; (*b*) J. H. Hartley, T. D. James and C. J. Ward, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3155.

7 N. J. Cusack, G. Shaw and F. I. Logemann, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2316.

