BULLETIN

OF THE

KOREAN CHEMICAL SOCIETY

ISSN 0253-2964
Volume 20, Number 2

BKCSDE 20(2) 125-256
February 20, 1999

Communications

Complexation and Transport of Zwitterionic Amino Acids by an Artificial Receptor

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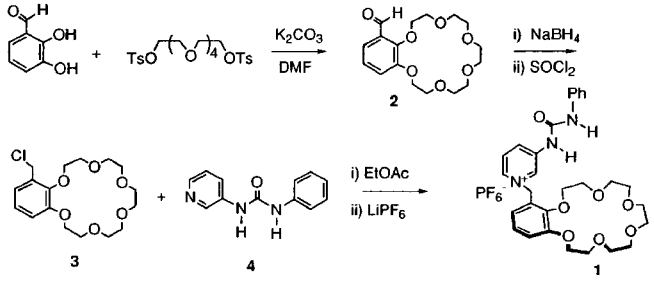
Received October 10, 1998

The development of artificial receptors for binding and transporting amino acids is a challenging problem because i) the amino acids exist as heavily solvated zwitterionic forms in neutral aqueous solution and ii) two oppositely charged functionalities (carboxylate and ammonium) were considerably stabilized by the mutual electrostatic forces. For a strong complexation and an efficient transport of zwitterionic amino acids, synthetic receptors should possess complementary binding sites for both ammonium and carboxylate functionalities. In this sense, a few elegant examples were reported by Rebek,[1] de Mendoza,[2] Schmidtchen,[3] and other groups.[4] We herein describe a receptor that binds to zwitterionic α-amino acids and efficiently transports them through a liquid membrane, $CHCl_3$. The new receptor **1** consists of two subunits, benzo-18-crown-6 and urea functionalities, for binding the ammonium and carboxylate groups of zwitterionic α-amino acids, respectively.

The synthesis of **1** is outlined in Scheme 1. Coupling of 2,3-dihydroxybenzaldehyde with pentaethylene glycol di-*p*-toluenesulfonate under basic conditions (K_2CO_3/DMF, ~80 °C, 76% yield) gave 3′-formylbenzo-18-crown-6 (**2**) which was reduced ($NaBH_4$/EtOH, rt, 93% yield), then treated with $SOCl_2$ ($CHCl_3$, reflux, 91% yield) to afford 3′-(chloromethyl)benzo-18-crown-6 (**3**). Reaction of the compound **3** with urea **4** (EtOAc, reflux), followed by anion exchange ($LiPF_6$/H_2O) provided the receptor **1**[5] in a 65% yield.



Scheme 1

Even though the receptor **1** has an ionic nature, it is highly soluble in $CDCl_3$, but not soluble in water. The amino acid-binding property of **1** was therefore examined by solid-liquid and liquid-liquid extractions at 24±1 °C. The solid-liquid extractions was performed by employing a $CDCl_3$ solution of **1** (5 mM, 0.6 mL) and excess solid ***L***-amino acids (10 mg), and the liquid-liquid extractions by mixing a $CDCl_3$ solution of **1** (5 mM, 1 mL) and an aqueous solution of ***L***-amino acids (100 mM, 1 mL). The amounts (±0.05 equiv.) of amino acids in $CDCl_3$ were determined by the ^1H NMR integration and the results are summarized in Table 1.

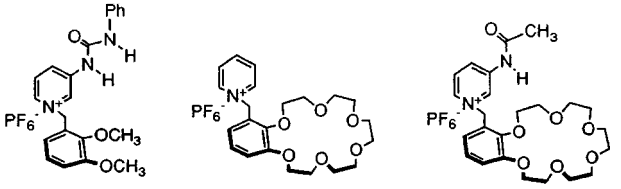
As shown in Table 1, our new receptor **1** extracted highly efficiently the amino acids with nonpolar side chains such as Phe, Ile, Leu, and Trp. For a comparison, de Mendoza's receptor, containing azacrown ether, guanidium, and naphthyl moieties as the binding sites, extracted ~0.4 equivalents of *L*-Phe and *L*-Trp from aqueous into organic phase under the similar conditions (200 mM of amino acids in aqueous solution, 5.5 mM of receptor in CH_2Cl_2).[2] None of the amino

Table 1. Results (molar equivalent±0.05) of Solid-Liquid and Liquid-Liquid Extractions*

extraction	Phe	Ile	Leu	Val	Ala	Tyr,Ser,His,Asp
solid-liquid	0.95	0.91	0.86	0.85	0.74	~0
liquid-liquid**	0.90	0.84	0.79	0.55	0.22	~0

*All extractions were at least duplicated and errors were within 6%. Molar equivalents were measured on cooling the solutions down to -40 °C at which temperature the sharp well-resolved spectra were observed in all cases. A large amount (~0.8 equiv) of Trp was extracted in both extractions, but exact molar equivalent could not be determined since the ^1H NMR spectrum of the mixture was relatively broad in a wide temperature range (-40 to 25 °C). **The pH values in the aqueous solution remained constant (5.87±0.01) before and after extractions.

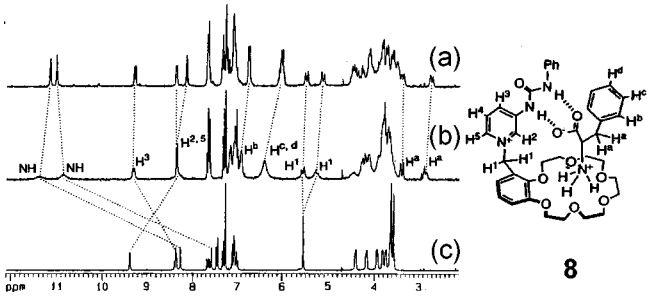
acids is soluble in $CHCl_3$, and thus extraction occurs by complexation. In the presence of amino acids in $CDCl_3$, for example *L*-Phe, the ^1H NMR spectrum of **1** is considerably changed as shown Figure 1: i) the urea NH resonances are highly downfield shifted ($\Delta\delta$ > 3 ppm), indicating a strong hydrogen bonding formation[6] between the urea of **1** and the carboxylate of amino acids, ii) the benzylic proton (H^1) signal is splitted into two sets of doublets due to the hindered rotation resulted from two point (urea-carboxylate and crown ether-ammonium) fixation, and iii) the H^2 and H^3 signals of the pyridinium ring are upfield and downfield shifted, respectively, due to the conformational reorientation of the urea carbonyl group upon complexation.



5 **6** **7**

In order to evaluate the individual contribution of the crown ether and urea subunits in **1** to the binding events, we prepared three reference compounds **5**-**7**. The crown ether subunit is removed in **5**, while the urea subunit is removed in **6** and replaced by the amide in **7**. Unlike the receptor **1**, none of these compounds **5**-**7** dissolved *L*-Phe and *L*-Ile in $CDCl_3$ at all. The results clearly indicate that two binding subunits of **1** are necessary for a strong complexation, and cooperatively involved in the hydrogen bonding with the ammonium and carboxylate of zwitterionic amino acids as shown in complex **8**.

The amino acid-transporting abilities of **1** through a $CHCl_3$ liquid membrane was carried out at 296±1 K using an apparatus (inner diameter 5.0 cm, each interphase area 39 cm^2) described by Rebek.[1] The amino acids (6.0 mM) of the source phase (20 mL of distilled water) were transferred into receiving phase (20 mL of distilled water) through a $CHCl_3$ membrane (20 mL) containing **1** (1.0 mM). The stirring rate of the organic phase was constantly controlled at a 200 rpm,[7] and aliquots (0.1 mL) of the receiving phase were removed in every 2hr. The amounts of the amino acids in the receiving phase was determined by UV-visible spectroscopy at 570 nm after ninhydrin treatments. The absolute and relative transport rates are summarized in Table 2.



8

Figure 1. ^1H NMR spectra of a) **1** (5 mM) at 23 °C, b) **1** (5 mM)+Phe (~5 mM) at 23 °C, c) **1** (5 mM)+Phe (~5 mM) at -40 °C in $CDCl_3$.

Table 2. Transport rates of amino acids by receptor **1** across a $CHCl_3$ membrane

amino acid	rate (±5%)*	relative rate
Phe	176	53
Trp	146	44
Ile	124	37
Leu	110	33
Val	60.5	18
Ala	13.3	4
Ser	3.31	1
Asp	< 0	0
His	< 0	0

*[mmol]×10^2 transported (h^{-1}·cm^{-2}·[**1**]$^{-1}$)

The overall transport efficiencies (Phe > Trp > Ile > Leu > Val >> Ala > Ser >> Asp, His) are consistent with the extraction results (Phe > Ile > Leu > Val > Ala >> Ser, Asp, His, Tyr). The transport of the amino acids (Ser, Asp, His) with polar side chains capable of hydrogen bonding formation are negligible as in extractions, indicating no appreciable polar interaction between **1** and the side chain. No special preference of the aromatic amino acids over aliphatic ones could be noticeable in both extraction and transport experiments. All of these observations imply that complexations between **1** and amino acids are mostly driven by a combination of the urea-carboxylate and the crown ether-ammonium hydrogen bondings. The observed selectivities therefore seem to reflect the lipophilicity of the amino acid side chains.[8]

In conclusion, a simple model receptor was described which binds to zwitterionic amino acids *via* multiple hydrogen bonding formation, and thus efficiently transports them across a $CHCl_3$ liquid membrane. Further study is underway to find a possible chiral discrimination of zwitterionic amino acids by chiral derivatives of the receptor **1**.

Acknowledgment. This work was financially supported in part by the the Korea Science and Engineering Foundation (Project No. 96-0501-04-01-03) and by the Organic Chemistry Research Center-KOSEF. We are grateful to Mr. Young Lag Cho for helping ^1H NMR experiments.

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5. mp 87 °C; IR (KBr) 3415, 3085, 2910, 1710, 1593, 1554, 1502, 1485, 1273, 1212, 1113, 842, 757, 730 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 3.55-3.65 (m, 10H), 3.74 (m, 2H), 3.81 (m, 2H), 3.94 (m, 2H), 4.16 (m, 2H), 4.41 (m, 2H), 5.55 (s, 2H), 6.97-7.13 (m, 4H), 7.29-7.33 (m, 2H), 7.43-7.47 (m, 2H), 7.58 (1H, s, NH) 7.65 (m, 1H), 8.26 (1H, s, NH), 8.34-8.39 (m, 2H), 9.38 (s, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 151.5, 151.4, 146.7, 140.7, 137.6, 136.8, 133.8, 133.0, 128.9, 127.5, 124.6, 123.7, 122.7, 119.5, 116.2, 72.9, 71.1, 70.5, 70.4, 70.1, 70.0, 69.3, 69.2, 68.0, 61.5, 54.4; Anal. calcd for $C_{29}H_{34}N_3O_7PF_6$: C, 50.95; H, 5.31; N, 6.15; Found: C, 50.99; H, 5.36; N, 6.09.
6. For the hydrogen bonding between urea and carboxylate, see: (a) Jeong, K.-S.; Park, J. W.; Cho, Y. L. *Tetrahedron Lett.* **1996**, *37*, 2795-2798. (b) Hughes, M. P.; Smith, B. D. *J. Org. Chem.* **1997**, *62*, 4492-4499 and references therein.
7. For the transport experiments, a Spinplus stirring bar (size 3/8 in x 3/8 in) and a MIRAK^{TM} Stirrer (Barnstead/Thermolyne Co.) were used. The pH values in the receiving and source phases remained constant (5.87±0.02) before and after transports. No sign of the *L*-Phe transport was observed in absence of **1**, or in the presence of the reference compounds **5**, **6**, and **7**.
8. For water-amino acid side chain interactions, see: (a) Wolfenden, R.; Andersson, L.; Cullis, P. M.; Southgate, C. C. B. *Biochemistry* **1981**, *20*, 849-855. (b) Wimley, W. C.; Creamer, T. P.; White, S. H. *Biochemistry* **1996**, *35*, 5109-5124.