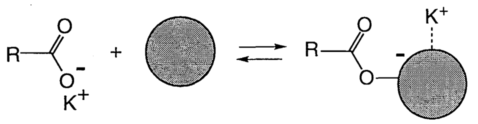
Efficient Transport of Aliphatic Amino Acids Mediated by Lanthanide Complex Carriers under Neutral Conditions

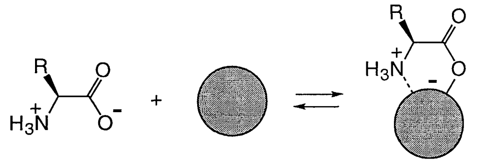
Hiroshi Tsukube,* Satoshi Shinoda, Jun'ichi Uenishi,† Mihoko Shiode,† and Osamu Yonemitsu†
Department of Chemistry, Faculty of Science, Osaka City University, Sugimoto Sumiyoshi-ku, Osaka 558
†*Department of Chemistry, Faculty of Science, Okayama University of Science, Ridai-cho, Okayama 700*

(Received July 31, 1996)

Lipophilic lanthanide tris(β-diketonates) effectively mediated transport of the aliphatic amino acids such as valine and leucine under neutral conditions, though conventional carriers rarely transported these zwitterions.

Transport of amino acids is one of the most fundamental biological processes, in which amino acids are transported as zwitterions under neutral conditions. A variety of carriers have been synthesized for amino acid derivatives, but most of them has been performed with single charged ammonium or carboxylate guests.[1] Since ammonium and carboxylate ions of the zwitterionic amino acids diminish the ability to interact with carriers, aliphatic amino acids were much difficult to be bound and transported under neutral conditions.[2-5]

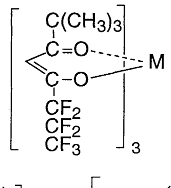

(Eq. 1)


(Eq. 2)

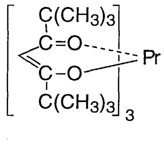
Here, we demonstrate that lipophilic lanthanide tris(β-diketonates) bind zwitterionic valine and leucine and efficiently transport them across a liquid membrane. Complexes of this type are known to coordinate with polar substrates[6] and are employed as NMR shift reagents as well as catalysts. We recently reported that lanthanide tris(β-diketonate) transported inorganic/organic anions *via* highly coordinated complexation (Eq. 1).[7,8] Since the resulting negatively charged complex can interact with cationic species through electrostatic attraction, the lanthanide tris(β-diketonates) can bind zwitterionic amino acids at two points (Eq. 2).[9]

We examined carrier activities of praseodymium complexes **1a** - **1d**, europium, holmium and copper complexes **2** - **4** and a binary carrier composed of crown ether **5** and surfactant **6**. The transport experiments were performed using a CH_2Cl_2 liquid membrane in which lanthanide complexes are distributed in a membrane and amino acids exist as zwitterions in an aqueous phase.[10] Table summarizes typical transport results using an aqueous mixture of five aliphatic amino acids as a source phase: serine (Ser); alanine (Ala); proline (Pro); valine (Val); and leucine (Leu). Interestingly, lanthanide complexes **1a**, **1c**, **2** and **3** having fluorinated ligands effectively mediated membrane transport of Val and Leu under neutral conditions, while praseodymium complexes **1b** and **1d** having non-fluorinated ligands produced insoluble materials and did not exhibit effective carrier activities. Their transport rates were satisfactorily high and reached a comparable level to that of crown ether-mediated K^+ cation transport.[11] They also transported aromatic amino acids. The transport rate of phenylalanine with complex **1a** was typically recorded as 4.9×10^{-6} mol/h which was 1.4 times higher than that of Leu.[12] Since pH change and β-diketonate leakage were rarely observed in either aqueous source or receiving phase, highly coordinated complexation between lanthanide tris(β-diketonate) and zwitterionic guest was involved (see Eq. 2). In contrast, copper complex **4** and binary carrier composed of **5** and **6** rarely carried zwitterionic amino acids. Thus, the central lanthanide cations were confirmed to play an important role in the transport of zwitterions.

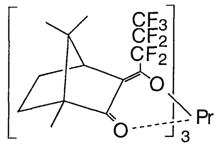
Liquid-liquid extraction experiments were carried out using the same five amino acids. Among these employed, Leu and Val were effectively extracted by lanthanide complexes. For example, praseodymium complex **1a** offered high extractability for them (conditions, see Ref.13): 27% for Leu;10% for Val; 8% for Pro; 4% for Ala and 5% for Ser.[13] No extraction of aliphatic amino acid was observed with copper complex **4** or binary carrier composed of **5** and **6**. Stoichiometry was determined using L-Leu and europium complex **2**. When the concentration of Leu in the aqueous phase increased, the mole ratio of Leu extracted to lanthanide complex increased and reached almost 1, indicating 1:1 complexation. After this extraction experiment, the CH_2Cl_2 phase exhibited characteristic induced CD signals at about 300 nm which responded to absorption bands of the original complex **2**. This probably indicates that the chiral Leu guest was tightly fixed



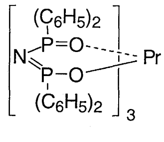
1a: M=Pr,
2: M=Eu, **3**: M=Ho



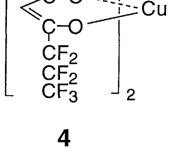
1b



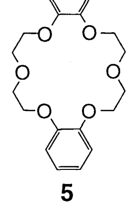
1c



1d



4



5

$(CH_3)_3N^+(CH_2)_{17}CH_3$
Cl^-

6

Table. Transport of zwitterionic amino acid by lanthanide complex

Carrier	Transport Rate (x 10^{-6} mol/h)[a]				
	Ser	Ala	Pro	Val	Leu
1a	<0.1	0.1	0.6	1.6	3.6
1b[b]	<0.1	<0.1	<0.1	<0.1	<0.1
1c	<0.1	0.1	0.4	1.6	2.9
1d[b]	<0.1	<0.1	<0.1	<0.1	<0.1
2	<0.1	0.1	0.4	1.4	3.2
3	<0.1	0.1	0.3	1.2	2.7
4[b]	<0.1	<0.1	<0.1	<0.1	<0.1
5 + 6	<0.1	<0.1	<0.1	<0.1	<0.1

[a] Amino acid, 0.2 mmol, each, in H_2O 5 ml (pH=6.0) // Lanthanide complex, 0.05 mmol in CH_2Cl_2 12 ml // H_2O 5 ml.

[b] Trace amount of precipitate appeared.

on the achiral lanthanide complex. When 5 mole% of **1a** or **2** was added to a $CDCl_3$ solution of N, N-dipropylalanine, ^{13}C NMR signals for -$\underline{C}H_2N\underline{C}H(CH_3)\underline{C}O$- disappeared. The lanthanide tris(β-diketonate) was, therefore, believed to bind amino acid at two points as shown in Eq. 2.

We demonstrated unique and effective carrier abilities of lanthanide tris(β-diketonates) for Leu and Val. Since these aliphatic amino acids were efficiently transported, the lanthanide complexes provide further design possibilities for new, specific sensing and separation systems for various amino acids and peptides of biological interest. The authors are grateful to Ms. M. Doe of the Analytical Center, Faculty of Science, Osaka City University for amino acid analysis.[14] This research was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas, "New Development of Rare Earth Complexes" (No. 0723026), from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

1 Review: H. Tsukube, *"Crown Ethers and Analogous Compounds,"* ed by M. Hiraoka, Elsevier, Amsterdam (1992), p. 100.

2 J. Rebek, Jr., B. Askew, D. Nemeth, and K. Parris, *J. Am. Chem. Soc.*, **109**, 2432 (1987).

3 Y. Aoyama, M. Asakawa, A. Yamagishi, H. Toi, and H. Ogoshi, *J. Am. Chem. Soc.*, **112**, 3145 (1990).

4 A. Galan, D. Andreu, A. M. Echavarren, P. Prados, and J. Mendoza, *J. Am. Chem. Soc.*, **114**, 1511 (1992).

5 M. T. Reetz, J. Huff, J. Rudolph, K. Töllner, A. Deege, and R. Goddard, *J. Am. Chem. Soc.*, **116**, 11588 (1994).

6 R.Hazama, K.Umakoshi, C.Kabuto, K. Kabuto, and Y. Sasaki, *J. Chem. Soc., Chem. Commun.*, **1996**, 15.

7 H. Tsukube, H. Shiba, and J. Uenishi, *J. Chem. Soc., Dalton*, **1995**, 181.

8 H. Tsukube, J. Uenishi, H. Shiba, and O.Yonemitsu, *J. Membrane Sci.*, **114**, 187 (1996).

9 Chiral lanthanide complexes of type **1c** extracted some aromatic amino acids enantioselectively. H.Tsukube, J. Uenishi, T.Kanatani, H.Itoh, and O.Yonemitsu, *J. Chem. Soc., Chem. Commun.*, **1996**, 477.

10 Lanthanide complexes as cation carriers: I. Willner, Y. Eichen, S. Sussan, and B. Shoham, *New J. Chem.*, **15**, 879 (1991): H. Tsukube, J. Uenishi, H. Higaki, and K. Kikkawa, *Chem. Lett.*, **1992**, 2307.

11 Transport rate of K^+ ion was recorded as 3.0 x 10^{-6} mol/h with binary carrier composed of **5** and **6** under similar conditions: $KClO_4$, 0.2 mmol in H_2O 5 ml // Carrier, 0.05 mmol in CH_2Cl_2 12 ml // H_2O 5 ml.

12 Enantiomer selective transport was attempted using chiral lanthanide complex **1c** but was not realized.

13 Extraction conditions: 5 amino acids, 0.01 mmol, each, in H_2O 2 ml // lanthanide complex, 0.02 mmol in CH_2Cl_2.

14 The concentration of each amino acid was determined based on amino acid analysis: Hitachi L-8500 Amino Acid Analyzer with a column (4.6 x 60 mm, #2622).