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A specific receptor of biological cystine polyion: distance-selective extraction and efficient chirality sensing with an ytterbium porphyrinate tweezer†

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An ytterbium porphyrinate dimer acts as a new class of tweezer-type receptor, which offers selective extraction of biological cystine polyion and chirality sensing with circular dichroism spectroscopy.

Cystine is a representative of biologically active polyions. It is comprised of two zwitterionic cysteins in a chiral disulfide skeleton and is significantly involved in glutathione synthesis, immune enhancement and brain neutrotransmitter processes. Although its polyionic and chiral structure is strongly recognized with these biological systems,[1] no synthetic receptor of such polyionic substrates has been developed.[2] Metallo–porphyrinate dimers are known as tweezer-type receptors effective for ditopic substrates.[3–6] Crossley *et al*.[3] and Hayashi *et al*.[4] linked two zinc porphyrinates with rigid spacers to form dimers which bound histidine ester, lysine ester and diamines through zinc–amine coordination. Nakanishi and coworkers[5] and Inoue and coworkers[6] further employed zinc porphyrinate dimers with flexible spacers as effective CD sensors for chirality determination of several amines. We recently demonstrated that lanthanide porphyrinate monomers extracted zwitterionic amino acids and were applied in CD chirality sensing.[7,8] Here, we first demonstrate that an ytterbium porphyrinate dimer specifically binds polyionic cystine and effectively senses its chirality with circular dichroism (CD) spectroscopy.[9]

Lanthanide porphyrinate tweezers **1** and **2** (Ln = Yb and Gd) were designed to form stable 1:1 complexes with ditopic substrates which are just long enough to bridge the two lanthanide centers (17.5 Å for **1a** and 20.0 Å for **2a**).[10–12] They have several outstanding receptor features: (1) each lanthanide porphyrinate binds zwitterionic amino acids *via* highly coordinated complexation; (2) the two lanthanide porphyrinates connected with a suitable spacer exhibit distance-selective binding of ditopic substrates; (3) supramolecular chirality is generated based on tweezer-type complexation between chiral substrate and achiral porphyrinate dimer; and (4) absolute configuration of the substrate can be sensitively detected by the CD method. The receptor functions of the lanthanide porphyrinate tweezers were characterized by liquid–liquid extraction of biological cystathionine, cystine and homocystine, and by subsequent CD measurements of the complexes. The three targeted polyions have two pairs of zwitterionic moieties and their spans were estimated as *ca*. 15.7, 17.3 and 20.1 Å, respectively.[10]

Ytterbium porphyrinate tweezer **1a** efficiently extracted cystine from an aqueous solution (pH = *ca*. 6) into a CH_2Cl_2 solution, while cystathionine, homocystine and methionine were modestly extracted (Fig. 1): cystathionine (6%) ≪ cystine (53%) ≫ homocystine (24%) > methionine (19%).[13] The pH values of the employed aqueous solutions were also recorded as *ca*. 6 after extraction experiments.[14] The fact that the extraction did not require interfacial H^+ transfer suggests a possiblity of highly coordinated complexation between each ytterbium porphyrinate and the zwitterionic moiety of the cystine as proposed earlier.[12,15] We calculated log *P* and log *D* at pH = 6.0 for each polyion using the PALLAS program,[16] which can be considered measures of hydrophobicity of the substrate. They increase in the order of cystathionine (log *P* = −1.75, log *D* = −5.1) < cystine (−1.16, −4.8) < homocystine (−0.45, −4.6) < methionine (−0.30, −2.5). Although several kinds of synthetic receptors were recently reported for zwitterionic amino acids, they were not applied for more hydrophilic polyions.[2] Remarkably, ytterbium tweezer **1a** extracted hydrophilic cystine more efficiently than hydrophobic homocystine and methionine, suggesting that cystine best fits this receptor and was most tightly bound. Probably, the two ytterbium porphyrinates of tweezer **1a** are arranged in a highly complementary manner to the two zwitterions of the cystine. Yterbium tweezer **2a** having a longer spacer favored longer homocystine, though its distance-selectivity was modest: cystathionine (5%) ≪ cystine (26%) < homocystine (34%) >

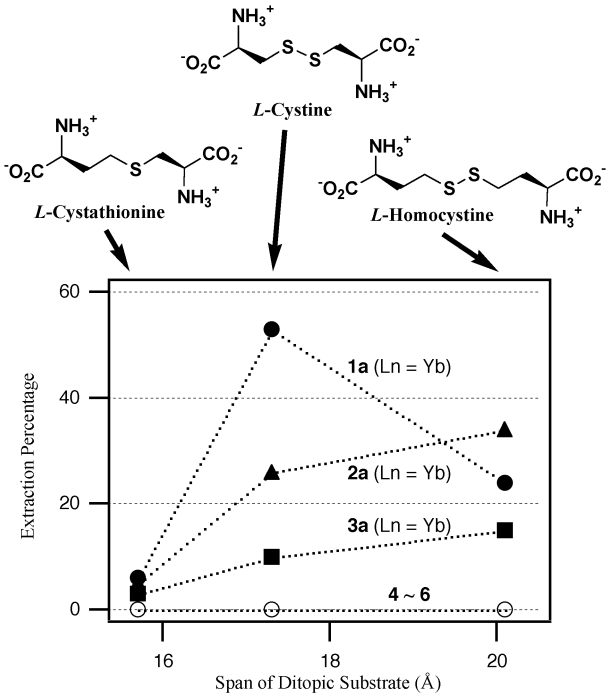

Fig. 1 Extraction of biological polyions. *Reagents and conditions*: polyion (cystathionine, cystine or homocystine) = 3.5×10^{-4} mol l^{-1} in H_2O (1.0 ml); porphyrinate dimer = 2.5×10^{-5} mol l^{-1}, porphyrinate monomer = 5.0×10^{-5} mol l^{-1} in CH_2Cl_2 (14.0 ml).

† Electronic supplementary information (ESI) available: synthesis of ytterbium porphyrinate tweezer **1a** and zinc analog **4**. See http://www.rsc.org/suppdata/cc/b2/b206708k/

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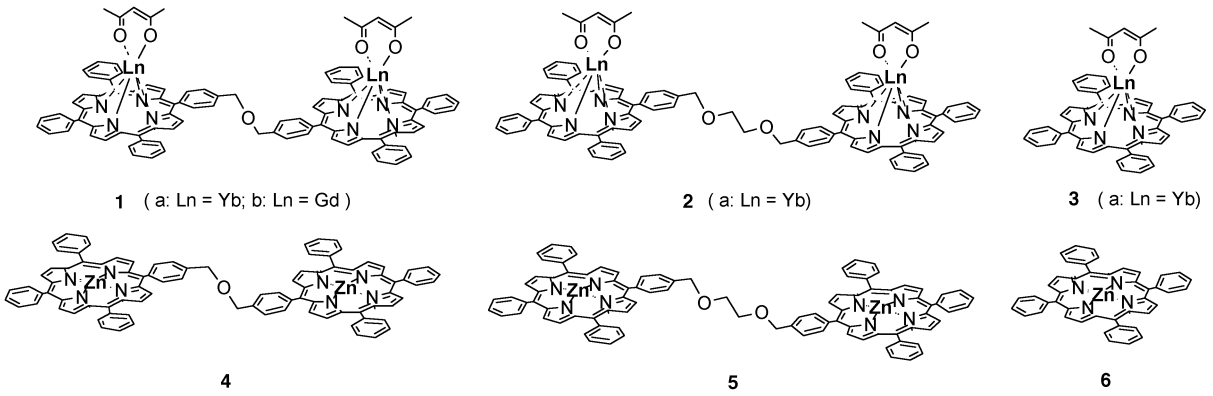

methionine (23%). Corresponding monomer **3a** extracted the examined polyions with modest efficiencies, and its extraction selectivity was apparently dependent on the substrate hydrophobicity: cystathionine (< 3%) < cystine (10%) < homocystine (15%) < methionine (24%). When gadolinium tweezer **1b** was employed, cystine was more effectively extracted than cystathionine or homocystine, but its distance-selectivity was lower than that with ytterbium tweezer **1a**: cystathionine (41%) < cystine (52%) > homocystine (44%) > methionine (28%). Since zinc porphyrinate dimers and monomer **4**–**6** rarely extracted polyionic substrates under the employed conditions (pH = *ca*. 6), the natures of spacer and lanthanide center should be considered in the design of specific lanthanide porphyrinate receptors of tweezer-type. Job plots indicated that lanthanide porphyrinate tweezers **1** and **2** mainly formed 1 : 1 (dimer : substrate) complexes with polyionic cystine and 1 : 2 complexes with zwitterionic methionine, while lanthanide porphyrinate monomer **3** formed a 2 : 1 (monomer : substrate) complex with cystine. Therefore, the intramolecularly cooperative action of the two lanthanide porphyrinates led to the specific extraction of highly hydrophilic cystine.

Achiral ytterbium porphyrinate tweezer **1a** exhibited intense CD signals around the Soret region upon extraction of chiral cystine (Fig. 2). The CD amplitudes were much larger than those with ytterbium porphyrinate dimer **2a** and monomer **3a**. Since cystathionine and homocystine polyions induced much weaker CD signals, the two porphyrinate moieties of tweezer **1a** were asymmetrically fixed upon multi-point binding of cystine. The cystine-induced CD spectra have complicated shapes, indicating the appearance of several overlapping signals at different positions.[17] Since the CD shape changed greatly as the cystine concentration increased, porphyrin–porphyrin aggregation, 1 : 1 and 2 : 1 complexation (dimer : cystine) and related equilibria steps must be involved. Fig. 2 also compares the induced CD signals observed with D- and L-cystine complexes,

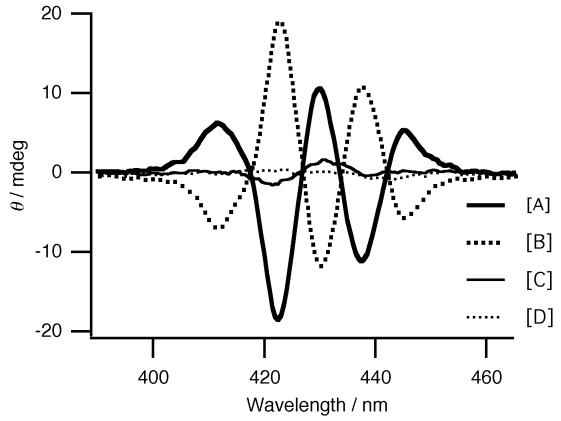

Fig. 2 CD spectra of ytterbium porphyrinates **1a**–**3a** in CH_2Cl_2 after shaking with aqueous solutions of L- and D-cystine. [A] **1a**–L-cystine, [B] **1a**–D-cystine, [C] **2a**–L-cystine, [D] **3a**–L-cystine. *Conditions*: see Fig. 1.

the signs of which were significantly dependent on the chirality of the employed substrates. This demonstrates that optically inactive porphyrinate tweezer **1** is applicable as an effective chirality sensor, which can specifically determine the absolute configuration of biological cystine using CD spectroscopy.

We successfully developed a new, effective receptor of cystine polyion by combining the tweezer-type molecular skeleton with a characteristic lanthanide porphyrinate. Since this offered highly efficient and selective recognition of polyionic cystine, a further sophistication of lanthanide prophyrinate receptor offers promising possibilities in the development of effective sensing, transport and separation systems for various biological polyions. The authors are grateful to Professors Kiyoshi Isobe and Isamu Kinoshita, and Ms Matsumi Doe of Osaka City University for valuable comments on CD measurements and polyion determinations.

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