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단 신

합성 수용체의 펩티드 결합선택성에 미치는 용매 효과

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Solvent Effect on Sequence-Selective Peptide Binding Properties of a Synthetic Receptor

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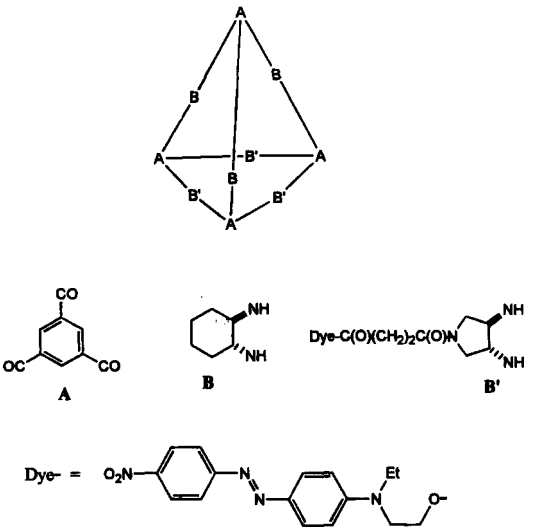
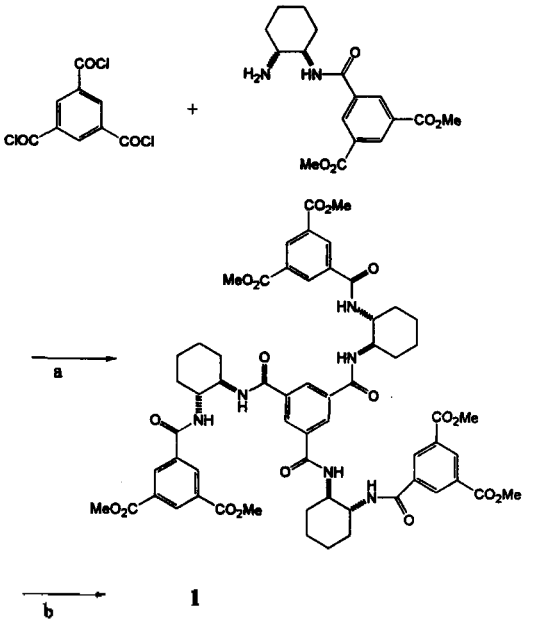
Molecular recognition has been widely studied because this would increase the understanding of basic principles on molecular recognition mechanisms seen in biological systems as well as the nature on non-covalent interactions between molecules, and lead to the selective catalysts, novel pharmaceuticals, novel analytic and separative tools.[1] One of the most exciting recent advances in molecular recognition is the development of synthetic receptors which bind polypeptides sequence-selectively.[2] Particularly, cyclooligomeric receptors derived from trimesic acid and 1,2-diamines showed high sequence-selective peptide binding properties.[3] In designing such receptors, workers typically sculpt preorganized binding sites to provide a complementary fit to desired polypeptide substrates. In such an effort, solvent effects are generally ignored except as they pertain to receptor solubility or a general driving force (*e.g.* hydrophobics) for substrate/receptor binding. In this report, solvent effect on sequence-selective binding properties of a known C_3-symmetric peptide-binding receptor (**1**)[4] is described. This study establishes that the peptide substrates preferentially bound by a receptor can be markedly different in different solvents, even when the different solvents are all chlorinated hydrocarbons.

< Structure of 1 >



RESULTS AND DISCUSSION

Synthesis of receptor (**1**) was straightforward and is outlined in *Scheme* 1. Synthesis of **1** begins with trimesic acid trichloride. A triple acylation with the 1-amino-2-(3',5'-dimethyloxycarbonylbenzoylamino)cyclohexane provided the hexamethylester. Ester hydrolysis and EDC coupling with pentafluorophenol led to the cyclization precursor, hexakis(pentafluorophenyl)ester. The final step was an intermolecular macrolactamization which used



Scheme 1. Synthesis of **1** a. DIPEA, THF (76%) b. i) pentafluorophenol/EDC (31.9%), ii) N-succynyl dye-(3R, 4R)-pyrrolidine diamine diTFA salt/DIPEA in THF (52%).

hexakis(pentafluorophenyl)ester and (3R,4R)-N-(Disperse Red I-succinyl)-pyrrolidine diamine di-TFA salts to close the macrocyclic ring. This cyclization provided the intensely red receptor **1** in 52% yield.

The polymer-supported, tag encoded tetrapeptide substrate library (R-AA3-AA2-AA1-polystyrene) was prepared by split synthesis using 15 different amino acid at each of three AA sites and the tripeptide chain terminated with 15 different acylating agent. Each spilit synthesis step was designated using particular combinations of 16 different tagging molecules as described previously.[5]

The peptide binding assay[6,7] were carried out by treating solutions of the dye-linked receptor (**1**) with an encoded library of 50,625 acylated tripeptides (R-AA3-AA2-AA1) on polystyrene synthesis beads in chosen solvents. The results in terms of the most commonly occurring residue at the most tightly bound substrates are shown in *Table* 1.

With receptor **1**, *Table* 1 indicates that the most tightly bound sequence had the same frequently occurring residues at the AA3 site in both $CHCl_3$ and TCE. However, variations of residues at R, AA1 and AA2 sites in the preferred substrate were found in both $CHCl_3$ and TCE. Thus **1** in $CHCl_3$ favored residue with R=Me, AA2=(L)Gln and AA 1=(L)Pro, but in TCE the preferred residue were R =Me_2N, AA2=(D)Ala and AA1=Gly.

The extent of these solvent effects on binding selectivity is further revealed by the number of occurrences of the less frequently found residues. While **1** is the most selective for the substrates having R=Me and Me_2N, AA2=(L)Gln and (D)Ala, and AA1=(L)Pro and Gly in $CHCl_3$ and TCE respectively, these same amino acids were found with virtually no statistical significance when the solvents were switched. For example, only 9% of the tightest-binding substrates in $CHCl_3$ had R= Me_2N, only 2% of the tightest-binding substrates in TCE had R=Me. Thus a residue that is strongly bound in one chlorohydrocarbon solvent can be only weakly bound in another.

To attribute these effects to a particular solvent property, the same solid phase color assay was employed with **1** in CH_2Cl_2 and $CH_2ClCHCl_2$. In CH_2Cl_2, the most tightly bound tripeptide sequence had the same most frequently occurring sequence as compared to that in $CHCl_3$ which has the sim-

Table 1. Residue selectivities of receptor (1) for sidechain-protected acylated tripeptides on a polystyrene support

Solvent	R (%[a])	AA3 (%[a])	AA2 (%[a])	AA1 (%[a])
$CHCl_3$	Me(45)	(D)Gln(29)	(L)Gln(59)	(L)Pro(57)
TCE	Me_2N(92)	(D)Gln(22)	(D)Ala(51)	Gly(43)
CH_2Cl_2	Me(43)	(D)Gln(27)	(L)Gln(58)	(L)Pro(59)
$CH_2ClCHCl_2$	Me_2N(91)	(D)Gln(20)	(D)Ala(49)	Gly(43)

[a]The percentage of the total bead that bound receptor and carried the indicated residue. The probability that each different would be found at each site for unselective binding is 6.7% (1/15).

ilar molecular size each other, but the very different sequence as compared to that in TCE which has the similar dielectric constant each other.[8] Also in $CH_2ClCHCl_2$, the most tightly bound tripeptide sequence had the same most frequently occurring sequence as compared to that in TCE which has the similar molecular size each other. Thus the data suggest that the size of solvent molecule play an important role in the observed differences in binding selectivities of **1**.

To get the insight on this notion, a CPK modeling study was conducted. The expected stable conformation of **1** revealed a interesting feature that it has a well-difined binding cavity large enough to accomodate a solvent molecule such as $CHCl_3$ and CH_2Cl_2, but small for TCE and $CH_2ClCHCl_2$. Previous studies have found that solvation of receptor's binding cavity with solvents play a major role in determining substrate binding selectivity of macrocyclic receptor.[9] Although it is not clear about the exact nature of the differences in peptide-binding selectivity of **1** in different solvents, the differences in the extents of solvation of receptor's binding cavity with solvents having different molecular sizes may play an important role. Presumably, the conformation of **1** is different from each other in different solvents due to different extents of solvation of receptor's binding cavity. Thus peptide binding properties of **1** change sensitively in solvents with different molecular sizes even when the solvents involved have similar structure.

Also, solvent effect on peptide binding properties of **1** is expected to be quite large when the solvents involved have different structure. To confirm this notion, the similar peptide binding assay were carried out in organic solvent such as THF and DMF. In THF, the most tightly bound tripeptide sequence [AcOM-(D)Asn-(L)Val-(L)Ser] was different from the most frequently occurring sequence in chlorinated hydrocarbons while **1** showed no binding property with tripeptide substrates in DMF. Thus solvent medium had significant influences on the peptide-binding properties of **1**.

In summary, this result make it clear that even subtle changes in the structure of solvent molecules can have a major effect on the binding of receptor with substrates. Thus in chemical operations where the nature of the solvent can be varied (*e.g.* chromatographic separations, selective chemical transformation), it is likely that significant changes in selectivity can be engineered by well-chosen alterations of the solvent medium. Also, this study demonstrate the power of directed screening of large chemical library[10] as a method to find novel substrate binding properties of receptors. Because such a library-based assay enables many binding experiments to be performed simultaneously, it is ideal for discovering binding phenomena about which little is known.

EXPERIMENTAL

Hexamethyl ester. To a solution of 0.15 g of (1R,2R)-2-(3',5'-methyloxycarbonyl benzoyl)aminocyclohexyl amine (0.137 mmol) and 0.13 mL of triethylamine (0.438 mmol) in 10 mL of DMA was added 35 mg of 1,3,5-benzenetricarbonylchloride (0.131 mmol). After stirring overnight at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 5% MeOH in methylene chloride to give an amorphous white solid (0.15 g, 98.5%): ^{1}H NMR ($CDCl_3$) δ (ppm) 1.44 (m, 4H), 1.94 (m, 2H), 2.10 (m, 1H), 2.20 (m, 1H), 3.87 (s, 6H), 3.94 (m, 2H), 7.10 (m, 1H), 7.30 (m, 1H), 8.25 (s, 1H), 8.55 (s, 2H), 8.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 166.82, 166.21, 165.00, 136.22, 134.64, 132.39, 131.94, 130.92, 129.44, 59.82, 54.88, 52.10, 32.10, 31.98, 25.42, 24.98; MS (FAB) *m/z* 2247 (M+1).

Receptor (1). To a solution of 0.14 g of hexamethyl ester (0.12 mmol) in 5 mL of THF, 3 mL of MeOH and 1 mL of water was added 0.84 mL of 1N NaOH solution. After stirring for 5 h at room temperature, the reaction mixture was acidified with 1N HCl solution and extracted with EtOAc (3×50 mL). The crude hexacarboxyl acid was dissolved in 3 mL of THF and 10 mL of

methylene chloride, and then 0.15 g of pentafluorophenol (0.84 mmol) and 0.16 g of EDC (0.84 mmol) were added. After stirring for 8 h at room temperature, all volatiles were removed at reduced pressure. The residue was purified by flash chromatography on silica gel using 20% acetone in methylene chloride to give hexakis (pentafluorophenyl)ester as an amorphous white solid (86 mg, 31.9%): ^{1}H NMR ($CDCl_3/CD_3OD$=1/1) δ (ppm) 1.40 (m, 4H), 1.83 (m, 2H), 2.11 (m, 1H), 2.25 (m, 1H), 3.97 (m, 2H), 7.09 (m, 1H), 7.32 (m, 1H), 8.30 (s, 1H), 8.75 (s, 2H), 8.97 (s, 1H).

A solution of 46 mg of hexakis(pentafluorophenyl)ester (0.025 mmol) and 7 mg of N-succynyl dye-(3R,4R)-prrrolidine diamine diTFA salt (0.075 mmol) in 10 mL of DMA was added to a solution of 0.15 mL of DIPEA (0.884 mmol) in 150 mL of THF at room temperature for 24 h by syringe pump. After stirring for additional 8 h, all volatiles were removed at redued pressure. The residue was purified by flash chromotography on silica gel using 10% MeOH in methylene chloride to give an amorphous red solid. (24 mg, 52%): ^{1}H NMR ($CDCl_3/CD_3OD$=1/1) δ(ppm) 8.18 (m, 4H), 8.00 (s, 1H), 7.74 (m, 4H), 6.71 (d, J=9.0 Hz, 2H), 4.68 (m, 2H), 4.20 (m, 2H), 3.95 (m, 4H), 3.62 (m, 2H), 3.42 (m, 2H), 2.52 (m, 4H), 1.95 (m, 2H), 1.70 (m, 2H), 1.32 (m, 4H), 1.12 (m, 3H); ^{13}C NMR ($CDCl_3/CD_3OD$=1/1) δ (ppm) 172.83, 170.12, 166.37, 156.64, 151.23, 147.11, 143.51, 134.47, 134.33, 133.70, 128.27, 126.04, 122.31, 111.19, 61.39, 61.29, 53.18, 46.85, 46.81, 45.43, 32.04, 31.76, 31.64, 28.46, 28.30, 28.18, 24.56, 24.40, 11.89; IR (neat) 3321, 2874, 1722, 1674, 1573 cm^{-1}; MS (FAB) *m/z* 2459 (M+1).

Procedure of the binding assay. 100 mg of the substrate library (~10^6 beads) were agitated for 48 h with the dye-linked receptor at a concentration of $30-40\times10^{-6}$ M in chosen solvents. At this point 0.1% of the beads had taken on the deep red coloration of the dye, Disperse Red I. Fifty of these beads were then picked and the structures of their associated substrates were determined using electron-capturing gas chromatography decoding as described previously.[6]

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   Our polymer-supported, encoded substrate library has the general structure:
   Polystyrene-$CH_2NHCO(CH_2)_5NH$-AA_1-AA_2-AA_3-C(=O)R
   where AA_n represents any one of the following fifteen amino acids: Gly, D-Ala, L-Ala, D-Ser(OtBu), L-Ser(OtBu), D-Val, L-Val, D-Pro, L-Pro, D-Asn (N-Tr), L-Asn(N-Tr), D-Gln(N-Tr), L-Gln(N-Tr), D-Lys(N-Boc), L-Lys(N-Boc) and R represents any one of the following fifteen groups: methyl (Me), ethyl (Et), isopropyl (iPr), t-butyl (t-Bu), neopentyl (neoPe), trifluoromethyl (TFM), isobutyl (iBu), methoxymethyl (MOM), acetoxymethyl (AcOM), cyclopropyl (cPr), cyclobutyl (cBu), cyclopentyl (cPe), phenyl (Ph), Morpholino (Mor), dimethylamino (Me_2N)
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