Highly Effective Binding of Phosphomonoester with Neutral Cyclic Peptides which Include a Non-natural Amino Acid

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Received May 2, 1995 (Revised Manuscript Received June 26, 1995)

Receptors for phosphoesters are currently attracting attention due to applications in transportation, catalytic hydrolysis, or complementary transition states.[1,2] When binding phosphoesters with proteins, the phosphoesters are bound with cationic side chains and/or the backbone of proteins. The general strategy for designing phosphoester receptors is salt formation with cationic groups such as ammonium or pyridinium.[1] However, there is no report, to our knowledge, about peptide receptors which bind with phosphoesters only *via* hydrogen bonds to the backbone, namely the amide hydrogen atoms. For this purpose, precise positioning amide groups in a peptide receptor are required. We synthesized cyclic peptides which consist of alternatingly natural and non-natural amino acids; 3-aminobenzoic acid (Aba) was used as the latter species to orient the amide group correctly around the phosphoesters. We now report that in the cyclic peptides *cyclo*(−AA-Aba−)$_n$ (Figure 1; AA denotes an amino acid), a hexapeptide, *cyclo*(−Ala-Aba−)$_3$, was found to be a highly efficient receptor for disodium 4-nitrophenyl phosphate despite the neutral peptide.

The synthesis of the cyclic peptide is summarized in Scheme 1. Since N-terminal free aminobenzoic acid derivatives such as H-Aba-OPac (Pac is the phenacyl group, $C_6H_5COCH_2-$) are easily oxidized under normal preparative conditions, dipeptides such as Boc-AA-Aba-OPac provide strength against oxidation and were initially prepared by the coupling of Boc-AA-OH with H-Aba-OPac·HCl using 1,3-dicyclohexylcarbodiimide (DCC). The Pac group was deprotected with zinc powder in acetic acid to yield Boc-AA-Aba-OH. Stepwise coupling of the Boc-AA-Aba-OH was afterwards performed by using the [(benzotriazol-1-yl)oxy]tris(dimethylamino)-

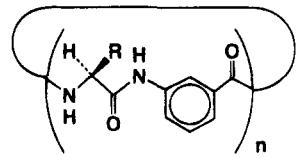
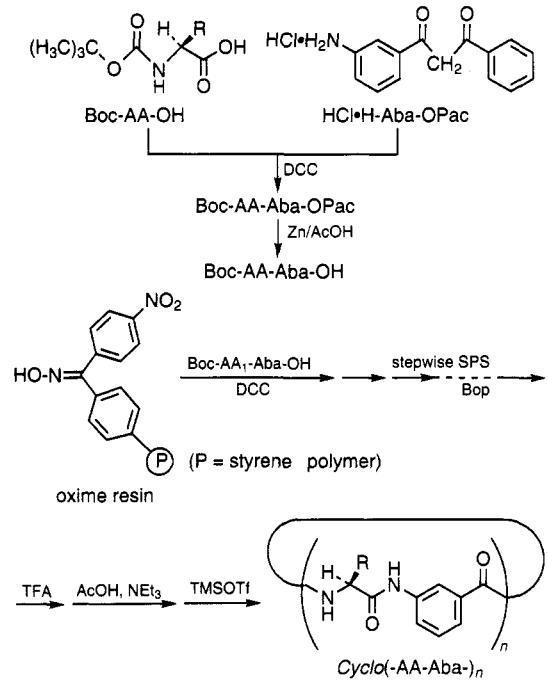


Figure 1. *Cyclo*(−AA-Aba−)$_n$.

Scheme 1



phosphonium hexafluorophosphate (BOP) method on a 4-nitrobenzophenone oxime resin, which was prepared according to the report of Nishino and Mihara *et al.*[3] Triethylamine (2 equiv) and acetic acid (2 equiv) in DMF were added to TFA·H-(AA-Aba)$_n$-resin (TFA is trifluoroacetic acid). Cyclization was performed on the resin to produce the cyclic peptides by shaking the mixture for 24 h. Removal of protecting groups was performed with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in TFA. They were identified from the NMR measurements and the mass spectroscopy data.[4]

Binding experiments were performed by titrations with electronic spectra. When the cyclic peptide, *cyclo*(−Ala-Aba−)$_3$, was added to disodium 4-nitrophenyl phosphate in DMSO, the electronic spectra dramatically changed as shown in Figure 2; the absorbance of the phosphomonoester at 314 and 436 nm increased and decreased, respectively, with an isosbestic point at 373 nm. The spectral changes were found to be due to the 1:1 complex formation of the peptide with the phosphomonoester, and the equilibrium constant was calculated from these changes (Table 1).[5] The equilibrium constants remarkably depended on the number of amino acids which cosntitute the cyclic peptides; they are large in the hexapeptides such as *cycloc*(−Ala-Aba−)$_3$, *cyclo*(−Ser-Aba−)$_3$, and *cyclo*(−Ser(Bzl)-Aba−)$_3$ (Bzl is a benzyl ether group). The largest binding contstants are observed in *cyclo*(−Ala-Aba−)$_3$. The equilibrium constants for *cyclo*(−Ser-Aba−)$_3$ and *cyclo*(−Ser(Bzl)-Aba−)$_3$ are somehow smaller, but they even maintain the order of 10^5. The larger peptides exhibit smaller equilibrium con-

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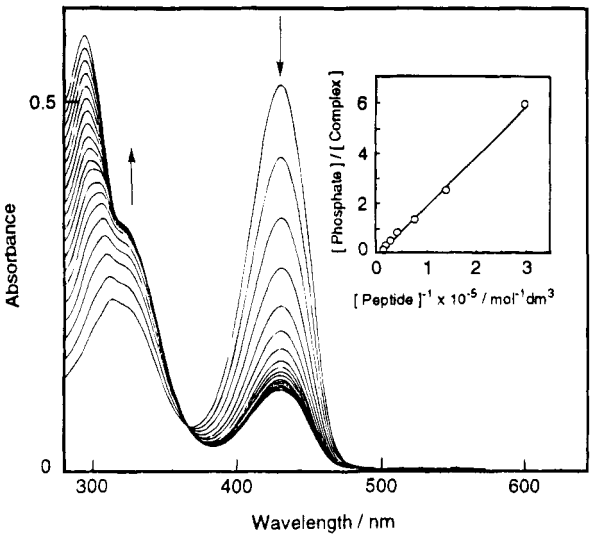


Figure 2. UV–vis spectral changes in disodium 4-nitrophenyl phosphate (4.0×10^{-5} mol dm^{-3}) by adding *cyclo*(–Ala-Aba–)$_3$ in DMSO at 298 K. Inset: analysis of the data according to the equation in ref 5.

Table 1. Binding Constants (K) for Receptors with Disodium 4-Nitrophenyl Phosphate in DMSO at 298 K

receptor	no. of amino acids	K/mol^{-1} dm^3
cyclo(–Ala-Aba–)$_3$	6	$1.2 (\pm 0.02) \times 10^6$
cyclo(–Ser(Bzl)-Aba–)$_3$	6	$4.8 (\pm 0.3) \times 10^5$
cyclo(–Ser-Aba–)$_3$	6	$2.5 (\pm 0.1) \times 10^5$
cyclo(–(Ala-Aba)$_2$–Phe-Aba-Ser(Bzl)-Aba–)	8	$2.0 (\pm 0.5) \times 10^4$
cyclo(–Ala-Aba-Ser(Bzl)-Aba-Phe-Aba-Ala)$_2$	14	$6.8 (\pm 0.8) \times 10^3$
Boc-(Ala-Aba)$_3$-OPac	6	$8.1 (\pm 1.0) \times 10^2$
Boc-Ala-Aba-OPac	2	<10

stants. For example, the binding constant with an octapeptide, *cyclo*(–(Ala-Aba)$_2$-Phe-Aba-Ser(Bzl)-Aba–), decreased to about 1/10–1/10^2 of the hexapeptide values. The factor whether the peptide is cyclic or noncyclic is important for the binding constants. The binding constant for the noncyclic peptide, Boc-(Ala-Aba)$_3$-OPac, remarkably decreased to about 1/10^3 of *cyclo*(–Ala-Aba–)$_3$. As expected, Boc-Ala-Aba-OPac did not bind with the phosphomonoester.

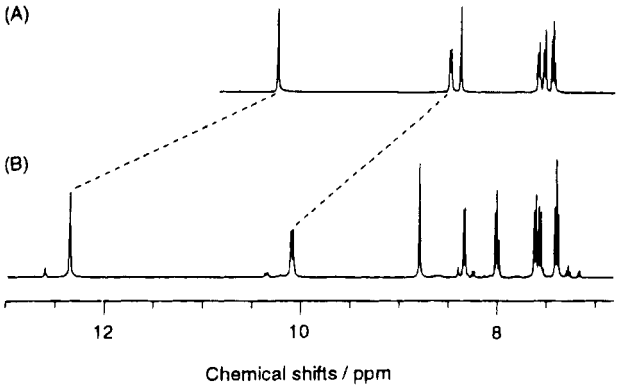
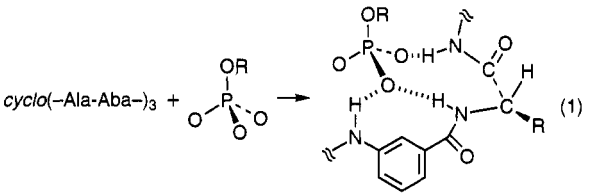


Figure 3. ^{1}H NMR spectra of the amide and phenyl parts of *cyclo*(–Ala-Aba–)$_3$ without (A) and with (B) an equimolar amount of disodium 4-nitrophenyl phosphate added to DMSO-d_6 at 298 K.

In order to obtain information about the interaction mode, we performed ^{1}H NMR experiments for *cyclo*(–Ala-Aba–)$_3$ which exhibits the highest binding constant (Figure 3). Three amide protons for Ala and Aba in the peptide show two single peaks at 8.47 and 10.23, respectively, in DMSO-d_6. This suggests that the cyclic peptide has C_3-symmetry. When disodium 4-nitrophenyl phosphate was added to the solution, the peaks for the amide protons drastically moved downfield to 10.08 and 12.36, respectively. The results revealed that *cyclo*(–Ala-Aba–)$_3$ did bind with the phosphomonoester *via* hydrogen bonds between every amide proton of the backbone and phosphate oxygens. Accordingly, the cyclic peptide was strongly suggested to bind with the phosphomonoester *via* six-point hydrogen bonding (eq 1).[6] Further investi-



gation using NMR measurements and molecular mechanics (MM) calculations is in progress.

We consider that the introduction of a non-natural and rigid amino acid to the backbone of the cyclic peptides resulted in the appearance of molecular recognition. Furthermore, it is felt that this group of cyclic peptides holds considerable potential for catalytic activity, and this is currently being investigated. We postulate that this strategy may lead us to a general method for designing functional peptides.

Acknowledgment. This work was funded in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

JO9508098

(4) *Cyclo*(–Ala-Aba–)$_3$: ^{1}H-NMR δ (400 MHz, DMSO-d_6) 10.23 (3H, s, amide(Aba)), 8.47 (3H, d, amide(Ala), $J_{HN\alpha}$ = 7.32 Hz), 8.37 (3H, s, 2-phenyl), 7.58 (3H, d, 4-phenyl), 7.52 (3H, d, 6-phenyl), 7.43 (3H, m, 5-phenyl), 4.64 (3H, m, CHα(Ala)), 1.45 (9H, d, CH$_3$(Ala)); MS *m/e* 572 (M$^+$). *Cyclo*(–Ser(Bzl)-Aba–)$_3$: ^{1}H-NMR δ (400 MHz, DMSO-d_6) 10.39 (3H, s, amide(Aba)) 8.54 (3H, s, 2-phenyl(Aba)), 8.40 (3H, d, amide(Ser)), 7.53 (3H, d, 4-phenyl(Aba)), 7.50 (3H, d, 6-phenyl(Aba)), 7.45 (3H, m, 5-phenyl(Aba)), 7.27 (15H, phenyl(Bzl)), 4.90 (3H, d, CHα(Ser)), 4.56 (6H, s, CH$_2$(Bzl)), 3.88 (6H, d, CH$_2$(Ser)); MS *m/e* 890 (M$^+$). *Cyclo*(–Ser-Aba–)$_3$: ^{1}H-NMR δ (400 MHz, DMSO-d_6) 10.37 (3H, s, amide(Aba)), 8.67 (3H, s, 2-phenyl), 8.15 (3H, d, $J_{HN\alpha}$ = 7.69 Hz, amide(Ser)), 7.55–7.42 (9H, m, 4, 5, 6-phenyl), 5.13 (3H, t, J = 5.86 Hz, OH), 4.73(3H, q, CHα(Ser)), 3.88(6H, br, CH$_2$(Ser)); MS *m/e* 619 (M$^+$). *Cyclo*(–(-Ala-Aba-)$_2$-Phe-Aba-Ser(Bzl)-Aba–): ^{1}H-NMR δ(400 MHz, DMSO-d_6) 10.33–10.06 (4H, m, amide(Aba)), 8.74 (1H, d, $J_{HN\alpha}$ = 7.33 Hz, amide(Phe)), 8.61 (1H, d, $J_{HN\alpha}$ = 9.28 Hz, amide(Ala)), 8.59 (1H, d, $J_{HN\alpha}$ = 7.84, amide(Ser)), 8.49 (1H, d, $J_{HN\alpha}$ = 6.83, amide(Ala)), 8.37–8.11 (4H, m, 2-phenyl(Aba)), 7.97–7.53 (12H, m, 4, 5, 6-phenyl(Aba)), 7.53–7.40 (5H, m, phenyl(Phe)), 7.35–7.18 (5H, m, phenyl(Bzl)), 4.85–4.80 (2H, m, CHα(Ser), CHα(Phe)), 4.63–4.56 (2H, m, CHα(Ala)), 4.56 (2H, s, CH$_2$(Bzl)), 3.85–3.84 (2H, br, CH$_2$(Ser)), 3.23–3.15 (2H, m, CH$_2$(Phe)), 1.44 (6H, d, CH$_3$(Ala)); MS *m/e* 944 (M$^+$). *Cyclo*(–Ala-Aba-Ser(Bzl)-Aba-Phe-Aba-Ala)$_2$: ^{1}H-NMR δ (400 MHz, DMSO-d_6) 10.23–10.16 (6H, m, amide(Aba)), 8.68–8.26 (8H, m, amide(Ala, Ser, Phe)), 8.21–8.06 (6H, m, 2-phenyl(Aba)), 7.68–7.41 (18H, m, 4, 5, 6-phenyl(Aba)), 7.40–7.33 (10H, m, phenyl(Phe)), 7.29–7.18 (10H, m, phenyl(Bzl)), 4.84–4.82 (4H, m, CHα(Ser, Phe)), 4.57 (4H, s, CH$_2$(Bzl)), 4.49–4.35 (4H, m, CHα(Ala)), 3.86 (4H, br, CH$_2$(Ser)), 3.19–3.08 (4H, m, CH$_2$(Phe)), 1.37–1.31 (12H, m, CH$_3$(Ala)).

(5) The binding constants (K) were obtained from absorbance changes (ΔA) at 436 nm according to [phosphate]/[complex] = (1/K)-[peptide]$^{-1}$, where [phosphate] = [phosphate]$_{total}$ – [complex], [peptide] = [peptide]$_{total}$ – [complex], and [complex] = ($\Delta A_{obsd}/\Delta A_{sat}$)[phosphate]$_{total}$.

(6) The absorption of the phosphomonoester in the UV–vis spectrum shown in Figure 2 is attributed to transition from a nonbonded orbital in the oxygen atoms of phosphate to π^*-orbital in the nitrophenyl ring. This transition is prevented by protonation of the phosphate oxygen atoms. It is strongly suggested by the NMR study that the cyclic peptide binds with the phosphomonoester *via* hydrogen bonding between the amide protons and the phosphate oxygen atoms. The hydrogen bonding should result in a similar effect to protonation for electronic transition of the phosphomonoester. Consequently, the spectral changes are proposed to be due to hydrogen bonding to the phosphate oxygen atoms.