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Design of an artificial molecular catalyst showing peptidase activity to the conserved sequence of HIV-1 envelope gp41

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Abstract

In designing the molecular catalyst having the ability to decompose the target molecule, two kinds of antigen recognition sites of the monoclonal antibodies were employed. One is anti-glycoprotein (gp) 41 monoclonal antibody raised against the highly conserved sequence (RGPDRPEGIEEEGGERDRD) of human immunodeficiency virus (HIV)-1 envelope. The conserved sequence is the molecule targeted in this study. Another is anti-hemin monoclonal antibody (1D3 antibody) which can firmly bind with porphine molecule. In these two monoclonal antibodies, the 16 mer peptide of the complementarity determining region (CDR)-1 in the light chain of the anti-gp41 monoclonal antibody displayed the strong binding affinity to the above targeted peptide molecule. As 17 mer peptide of the CDR-2 in the heavy chain of the 1D3 antibody had a strong interaction with porphyrin. The above 16 and 17 mer peptides were bridged via a spacer of –Gly–Pro–. The synthesized polypeptide could simultaneously uptake both porphyrin and targeted peptide. In addition, the polypeptide accelerated the decomposition of the targeted peptide by 6–14 fold, showing the peptidase activity as an artificial catalyst.

Keywords: Antibody; gp41; Complementarity determining region (CDR); HIV; Porphyrin

1. Introduction

During the past decade, studies on bispecific or chimeric antibody have been remarkably developed. Many methods to construct the bispecific antibody were reported in terms of gene engineering [1,2], hybridoma technique [3] and chemical recombination [4,5]. On the other hand,

Abbreviations: HIV: human immunodeficiency virus (or AIDS virus); 41S-2 antibody: anti-HIV-1 envelope gp41 antibody; 1D3 antibody: anti-hemin antibody; ELISA: enzyme-linked immunosorbent assay; PBS: phosphate-buffered saline; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCPP: *meso*-tetra(4-carboxyphenyl)porphine; CDR: complementarity determining region; CDRH-2: CDR-2 of heavy chain; CDRL-1: CDR-1 of light chain; Mn-MPMME: manganese mesoporphyrin conjugated with polyethylene glycol monomethyl ester; TCCP: *meso*-tetra(4-carboxyphenyl)porphine.

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the immunochemical features and the functions of complementarity determining region (CDR) of antibodies have been considerably revealed and then the CDR peptides or its chemically modified molecules have been synthesized as pharmacological agents [6–9].

Porphyrin and its derivatives are well-known as the effective medicinal agents against human immunodeficiency virus (HIV) and cancer. Anti-hemin (porphyrin) monoclonal antibody (1D3 antibody) has already been established and the sequence and the chemical features of the CDRs have been clarified by Uda et al. [10], Hifumi et al. [11], and Takahashi et al. [12], in which the CDR-2 of the heavy chain plays the main role to bind the porphyrins among six CDRs in the antibody.

Through recent AIDS studies, it has become clear that the establishment of vaccine against the HIV is extremely difficult, for the sequence of the envelope protein of the HIV frequently mutates even after the infection to human T cell. As the consequence, the development of anti-HIV drug is mainly directed to exploit the inhibitors of the enzymes such as reverse transcriptase, protease, etc., in the virion. Nonetheless, it has been well-known that there are some conserved sequences which never mutate in many HIV-1 strains. One of the sequence is present in glycoprotein 41 (gp41) in the envelope of the virion. It is the RGPDRPEGIEEEGGERDRD sequence which locates at the amino acid number 732–750 of HIV-1 and elicits the neutralizing antibody. As the gp41 molecule plays very important role in signaling the infection of HIV-1, the designing of the biological catalyst being able to decompose the gp41 molecule will give a new concept and technology in developing the drugs against HIV.

We have already established the monoclonal antibody (anti-HIV-1 envelope gp41 antibody or 41S-2) to the above sequence [13,14]. With the modification of these two monoclonal antibodies, 1D3 and 41S-2, we designed the artificial molecular catalyst as illustrated in Fig. 1. The molecule is composed of three kinds of molecular modules. First one is the molecular module A which incorporates the peptide antigen, RGPDRPEGIEEEGGERDRD. Second is the molecular module B being able to bind with porphyrin which acts as a catalytic site to accelerate the decomposition of the peptide antigen. Third is the spacer which bridges the molecular modules A and B. The designed molecule must be below 5000 in its molecular weight because, if over 5000, it gives a huge immunoresponse in human body, resulting anaphraxis shock, etc. by the repeated injection.

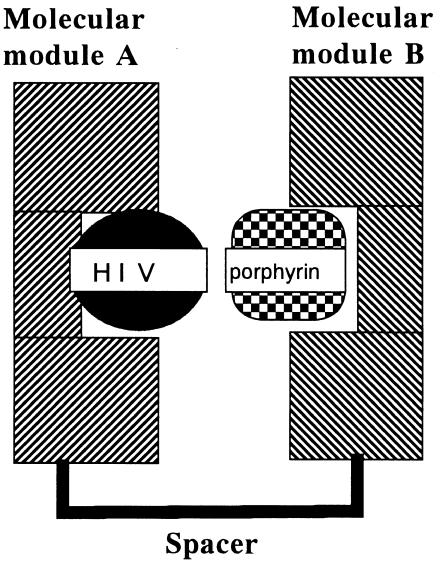

Fig. 1. The concept of the artificial molecular catalyst.

2. Experimental

2.1. Reagents

Monoclonal antibody: The monoclonal antibodies used in this study — 41S-2 and 1D3 — were mostly obtained as ascites fluid from *balb / c* mice. The antibodies were purified by salting out the ascites fluid with 33% ammonium sulfate and then passing it through diethylaminoethyl ion exchange chromatography. The purity was confirmed by sodium dodecyl sulfate

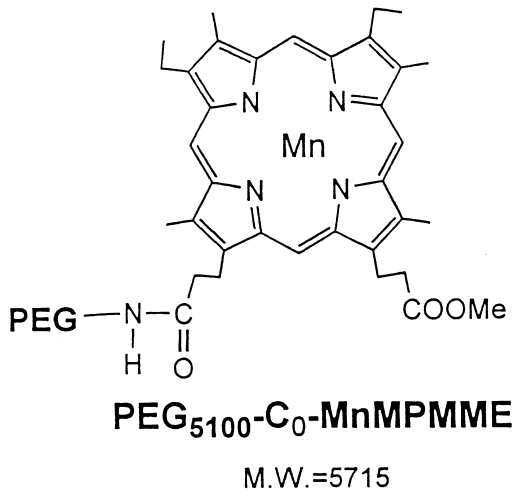

Fig. 2. The structure of Mn-MPMME.

poly-acrylamide gel electrophoresis (SDS-PAGE).

Mn-MPMME: Manganese-protoporphyrin conjugated with polyethylenglycol 5000 methyl ester was synthesized in accordance with the reference [15] and the structure (Fig. 2) was confirmed by NMR.

2.2. Purification of antibody and the separation to heavy and light chains

A total of 20 mg/ml of the antibody was dialyzed against 0.5 M Tris–HCl (pH 8.0) buffer for 2 days. The buffer containing 2 mM EDTA was exchanged three times during the dialysis. The antibody solution was concentrated to 2.5 ml by ultrafiltration (UFP1 TTK 24, Nihon Millipore, Yonezawa, Japan). To the concentrated solution, 0.275 ml of 100 mM dithiothreitol was added and the mixture was incubated at room temperature for 1 h with agitation. After the incubation, 0.275 ml of 210 mM iodoacetic acid was poured into the mixture and then the solution was allowed to stand at room temperature for 30 min with mixing. Then, 50 μl of 100 mM dithiothreitol was added and incubated for 15 min. Finally, the mixture was chromatographed on Sephadex G-25 and G-100 using 1 M propionic acid solution.

2.3. ELISA (enzyme-linked immunosorbent assay)

One hundred microliters of peptide antigen (10 μg/ml) dissolved in phosphate buffered saline (PBS) solution was poured into each well of 96 immunoplate (Nunc, Denmark) and incubated overnight at 4°C. Blocking was performed using 0.1% gelatin for 30 min at room temperature. After the plate was washed with PBS three times, 50 μl of the heavy and light chain and the 41S-2 antibody were added, followed by 1 h incubation at room temperature. After washing of the plate, 100 μl of the alkali phosphatase-labeled anti-mouse Ig antibody (Zymed, USA) was added and then the substrate reaction was carried out for color development. The absorption band at 405 nm was used for measuring the degree of enzymatic reaction by the immunoreader (InterMed NJ-2001).

2.4. Peptide synthesis

The peptides used in the experiments were synthesized by the Fmoc solid-phase method using an automated peptide synthesizer (Applied Biosystems 431A, USA). After deprotection of the resultant peptides from the resin, the peptides were purified by reverse-phase high performance liquid chromatography (RP-HPLC, Waters uBONDASPHERE C_{18} column, 17%–21% acetonitrile gradient for 20 min at flow rate 2 ml/min). The purity was confirmed 90%–97% as shown in Fig. 3. The peptides were identified by ion spray type mass spectrometry (API-III, Perkin-Elmer Sciex, Ontario, Canada).

2.5. Molecular modeling

The computational analysis of 41S-2 antibody was performed by use of a work station (Silicon Graphics, PA, USA). At first, the software of AbM from Oxford Molecular (Oxford, UK) was used to build up the three-dimensional molecule of the antibody using the amino acid sequences of its light and heavy chains. The

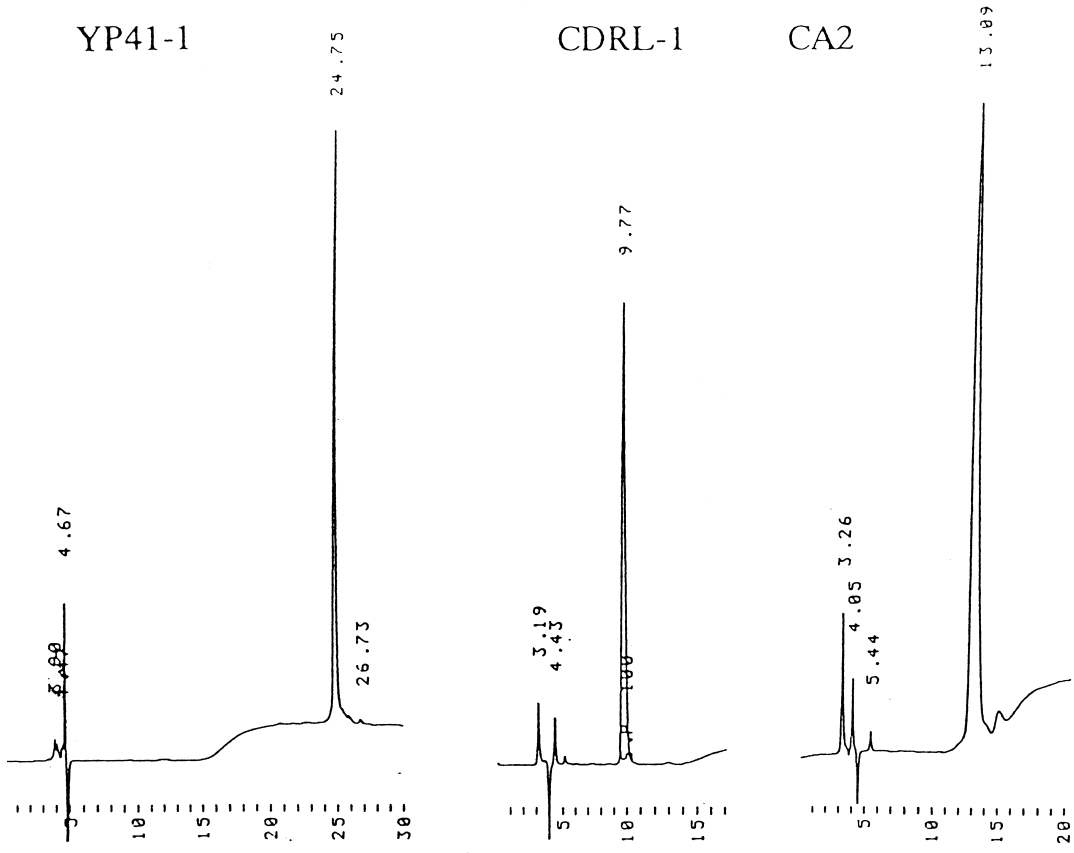

Fig. 3. Results of HPLC analysis for synthesized peptides.

software of Discover (Molecular Simulations, CA, USA) was used for the calculation of steric conformation of the designed molecule.

2.6. CD spectra

Samples (about 40 μM) were dissolved in 15 mM phosphate buffer (pH = 7.5), and spectra were recorded by using a computer-controlled Jasco J-700 CD spectropolarimeter, cylindrical cell having an optical path of 0.1 cm, resolution of 0.2 nm, and accumulation of 10.

3. Results and discussion

In order to investigate the recognition site of anti-HIV-1 envelope gp41 antibody (41S-2 antibody) and to clarify its chemical feature, ELISAs were performed to evaluate the apparent affinity constants of the 41S-2 antibody and its heavy and light chain with the peptide, YPRGPDR-PEGIEEEGGERDRD (YP41 peptide), coated on the immunoplate. Fig. 4 shows the results for the immunoreactions of these antibodies with YP41 peptide. The 41S-2 antibody displayed the highest affinity being about 4.2×10^{10} M^{-1}. That of the heavy and light chain was estimated to be 2.5×10^{8} M^{-1} and 8.3×10^{8} M^{-1}, respectively. The light chain binds with the YP41 peptide stronger than the heavy chain.

Messenger RNA was extracted from 5×10^{7} hybridoma cells and the nucleotide sequences of the heavy and light chains were determined. Table 1 represents the results for the nucleotide and the deduced amino acid sequences of the

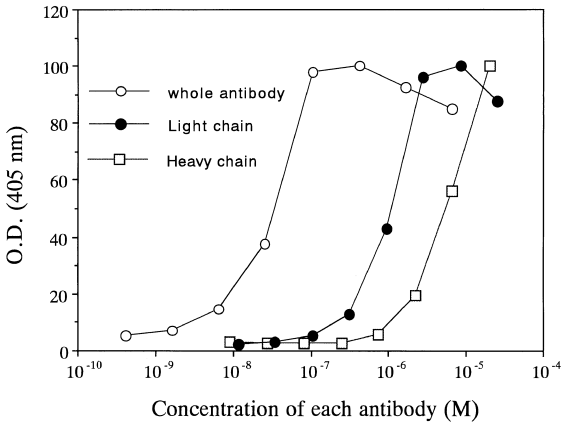

Fig. 4. The results of ELISA for 41S-2 antibody and its heavy and light chains.

CDRs of the light chain in the anti-gp41 antibody. The CDR-1 of the light chain (CDRL-1) has two characteristic features in the sequence. One is that three amino acid residues — serine (four residues), leucine (three residues) and tyrosine (three residues) — are the main in 16 amino acid residues. Another is the full sequence of amino acid residues to be allowed with maximum in CDRL-1 were inserted. In contrast, the sequence of CDRL-3 was forced to be shortened. This fact suggests that the CDRL-1 plays a main role in binding the antigen in the light chain.

Fig. 5 shows the conformation of the variable region of 41S-2 antibody, which was obtained by the molecular modeling. The white circle in the figure shows the main part of the CDRL-1 which projects the conformation to outside and much surfaces are exposed to the solvent in comparison with those of other CDRs, leading the easiness for the antigen to approach and bind to the CDRL-1.

Based on the above results, the 16 mer peptide of the CDRL-1 was synthesized and its immunochemical feature was investigated by using CD spectroscopy (Fig. 6). Each peptide of YP41 and CDRL-1 showed the gradual decreases from 240 nm with the decrease of wavelength. On the other hand, when both peptides were mixed at room temperature, the spectra largely changed soon and gave a minimum at 206 nm. The unordered structure may be formed with the strong interactions between YP41 and CDRL-1 peptides. These facts mean that CDRL-1 firmly binds with YP41 peptide.

Authors have already revealed that porphyrins firmly bind to the CDR-2 peptide (17 mer; RINPYNGATSYNQNFKD, CDRH-2) in the heavy chain of 1D3 antibody [10–12]. The affinity constant of CDRH-2 with *meso*-tetra(4-carboxy phenyl)porphine (TCCP) was determined to be $6.8 \times 10^7\ M^{-1}$, which is larger than the average value of the affinity constant (1/dissociation constant) of natural enzyme by 10–100 fold.

Table 1
Nucleotide sequence and its deduced amino acid sequences for CDRs of 41S-2 light chain

	nucleotide and amino acid sequences of each CDR
CDRL-1	R S S K S L L Y S N G N T Y L Y AGGTCCAGTAAGAGTCTCCTGTATAGTAATGGCAACACTTACTTGTAT
CDRL-2	R L F H L A CGGCTGTTCCACCTTGCC
CDRL-3	M Q H L E Y P Y T ATGCAACATCTAGAATATCCGTACACG

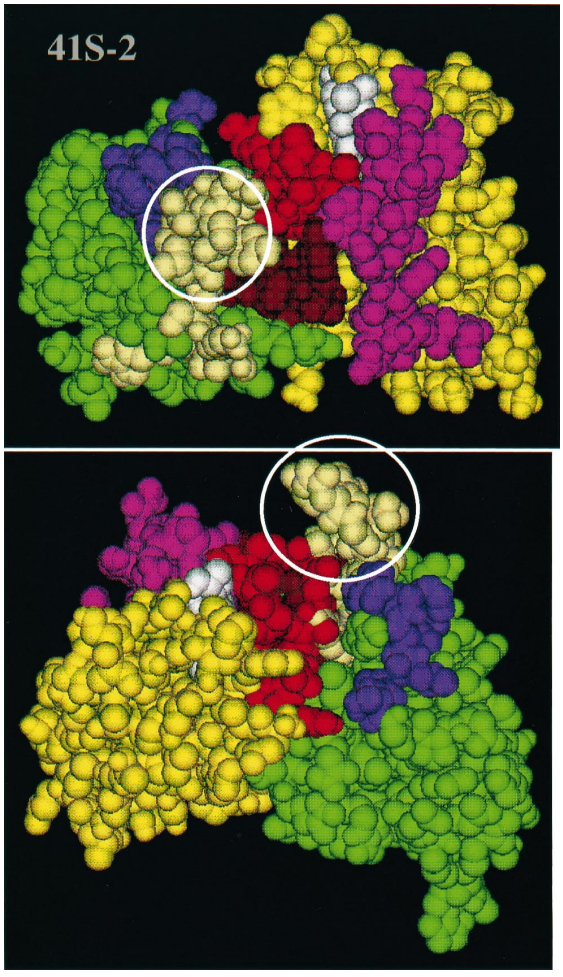

Fig. 5. Steric conformation of variable region of 41S-2 antibody. Upper photo: the heavy chain is on the right-hand side (the yellow color is the framework of heavy chain), while the light chain is on the left-hand side (the green color is the framework of the light chain). CDRL-1: pale yellow, CDRL-2: violet, CDRL-3: brown, CDRH-1: white, CDRH-2: pink, CDRH-3: red. Bottom photo: this view is rotated 90° around *X*-axis at first and then further rotated 90° around *Y*-axis from the upper photo. The white circle is the main part of CDRL-1.

From the above discussion and the facts, the most preferable amino acid sequence is the CDRL-1 of 41S-2 antibody for the module A and the CDRH-2 of 1D3 antibody for the module B. Hence, next step is to search the most appropriate spacer bridging the two modules. Computational calculations were performed to exploit the conformation of the designed polypeptide, [CDRL-1]-spacer-[CDRH-2]. Some spacers composed of di- and tri-peptides were applied to the computational calculations. Only when –Gly–Pro– was employed as a spacer, a steric space which has a room to incorporate both porphyrin and YP41 peptide could be generated as shown in Fig. 7. Although the molecular modeling is not perfect at present time, it suggests to synthesize the polypeptide, RSSK-SLLYSNGNTYLY-GP-RINPYNGATSYNQN-FKDH (CA2), which was prepared at one step synthesis by using the automated peptide synthesizer.

The uptake of porphyrin and YP-41 peptide with CA2 was measured by using CD spectrometry. Mn-MPMME was selected because of its water solubility and its well-defined property, in which manganese porphyrins caused an enhanced oxidation of aromatic hydrocarbons [15]. Furthermore, we reasoned that the PEG moiety on the compound was chemically stable and had no specific interaction with antibody under the condition of this study. Fig. 8a presents the spectra of a mixture of Mn-MPMME and CA2. In the spectrum of 0 h, a shoulder at around 225 nm and two minimums at around 195–200 nm were characteristic. The ‘simulation’ in the figure means the simulated spectrum that was calculated with the computer by assuming the ideal mixture state of 40 μM of Mn-MPMME and CA2, whose spectra had been taken prior to the above experiment. If there is no interaction in mixing of Mn-MPMME and CA2, the spectra must become identical with the simulation spectrum. However, these are completely different. Thus, the strong interaction must be generated just after the mixing of two compounds. Fig. 8b shows the spectra for a mixture of YP41 and CA2. The simulation and 0 h spectra were fairly identical. The spectra showed the gradual decrease from 250 to 200 nm and gave the minimum at around 200 nm. However, the 0-h spectrum largely changed after the 16-h incubation at room temperature. The minimum position markedly shifted to upward. This means that the interaction was gradually generated.

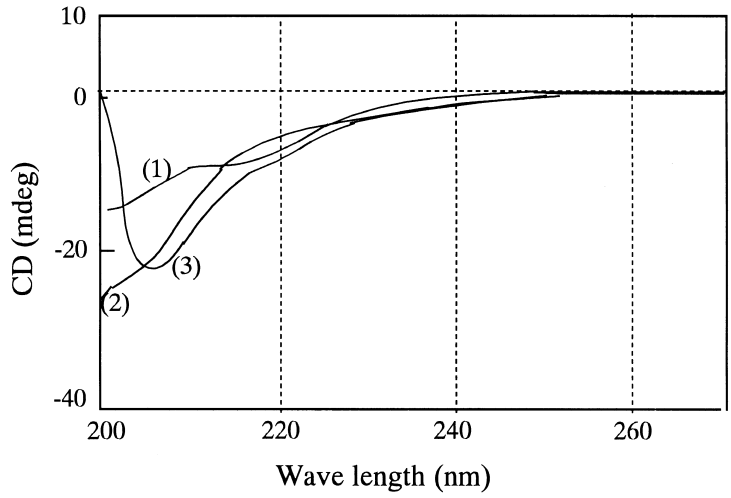

Fig. 6. CD spectra. Concentration of each peptide is 210 μM in phosphate buffer. (1) CDRL-1, (2) YP41, (3) CDRL-1 + YP41.

Fig. 8c shows the spectra when Mn-MPMME, YP41 and CA2 were simultaneously mixed. The spectrum at 0 h fairly changed from the simulated one. Furthermore, the shoulders of 195 nm caused by porphyrin and 220–230 nm caused by YP41 peptide were characteristically observed. These indicate that CA2 simultaneously uptakes two antigen, porphyrin and YP41, at same time. From the 16-h spectrum, it seems that the interacting conformation were gradually altered. In the case of the mixture of CA2 and YP41, the uptake of YP41 by CA2 is considered to be slow because both spectra at 0 h and the simulation are very similar. On the other hand, Mn-MPMME and CA2 had a rapid interaction each other. The simulation of the added

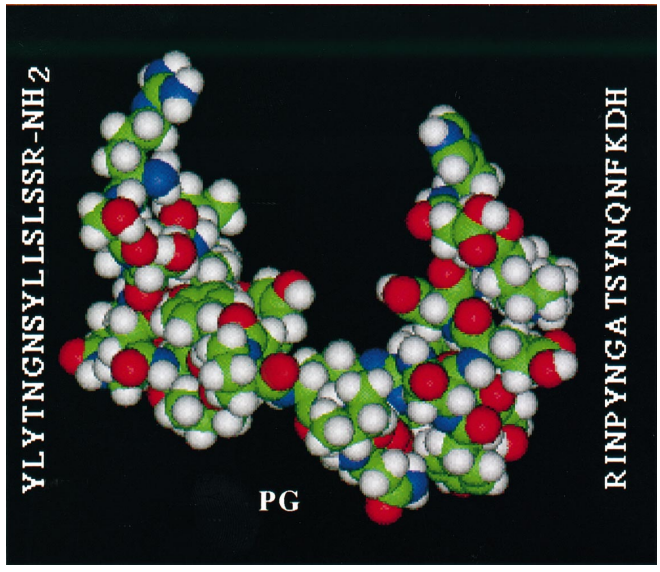

Fig. 7. Molecular modeling of the designed molecule. Hydrogen: white, nitrogen: blue, carbon: green, oxygen: red.

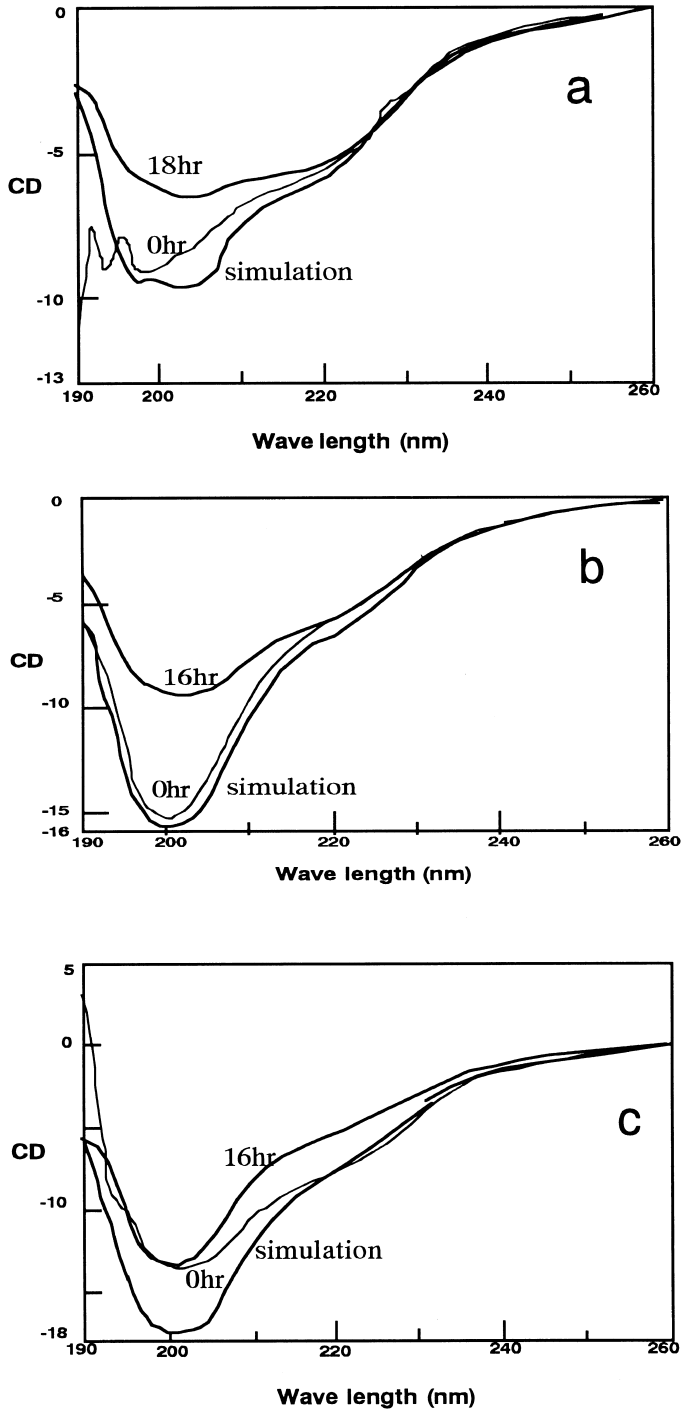

Fig. 8. CD spectra. Concentration: 40 μM for each molecule in any case. (a) Mixture of Mn-MPMME and CA2; (b) mixture of YP41 and CA2; (c) mixture of Mn-MPMME, YP41 and CA2.

spectra of 18 h in Fig. 8a and 16 h in Fig. 8b gave fairly identical with the spectrum of 16 h in Fig. 8c. This fact also supports that CA2 simultaneously uptakes two kinds of antigens.

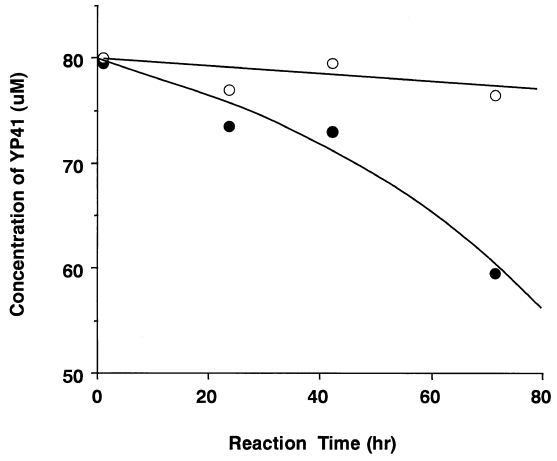

Fig. 9. Reaction change of YP41 by the artificial molecular catalyst, CA-2. (○) Without CA2 (control experiment). (●) With CA2. Reaction condition: CA2, 11.5 μM; YP41, 79 μM; Mn-MPMME, 2.29 μM in water at 62°C.

The catalytic feature of CA-2 was investigated with the above two antigens, porphyrin and YP41 peptide. Fig. 9 shows the time course of the catalytic reaction. In the experiment, YP41 peptide and Mn-MPMME were present at same time with or without CA2. The reaction was carried out at 62°C under the oxygen atmosphere. The concentration of YP41-1 peptide decreased with the time elapsed when CA2 was present in the reaction system. At the initial stage of the reaction, the decomposition was accelerated by about six-fold compared with the control experiment. The acceleration increased upto about 14-fold at 72 h. In the case that the porphyrin does not have Mn atom in the ring, no acceleration was observed. Therefore, the incorporated porphyrin in CA should become an active site. Considering all porphyrins work as an active site, the turnover of the decomposition of the peptide was 0.2/h, though the decomposition mechanism is not clarified at present time. The porphyrin molecule is assumed to hydrolyze the targeted peptide, YP41, into amino acids considering the results of HPLC. Thus, one porphyrin hydrolyzes the targeting peptide at the rate of 4.0/h because it has 20 peptide bonds in the sequence.

4. Conclusion

Though the catalytic activity is not so high at present time with the designed molecular catalyst, the acceleration of the decomposition against the targeted molecule was observed by 6–14 fold. By using the CDRs of the antibody which can uptake the several antigens simultaneously, a new biological catalyst could be fabricated. The results will give an insight to advance this new concept in developing the artificial molecular catalyst as medicinal drugs such as anti-viral, anti-cancer, etc.

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