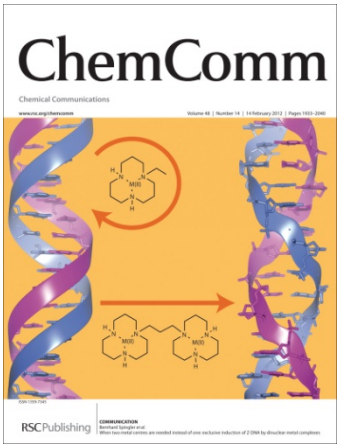

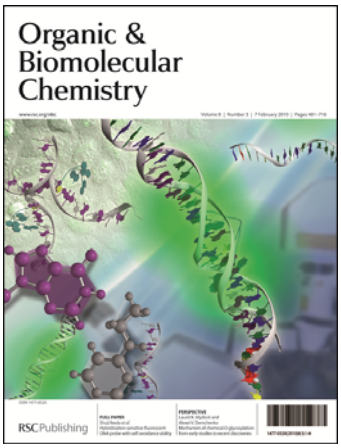

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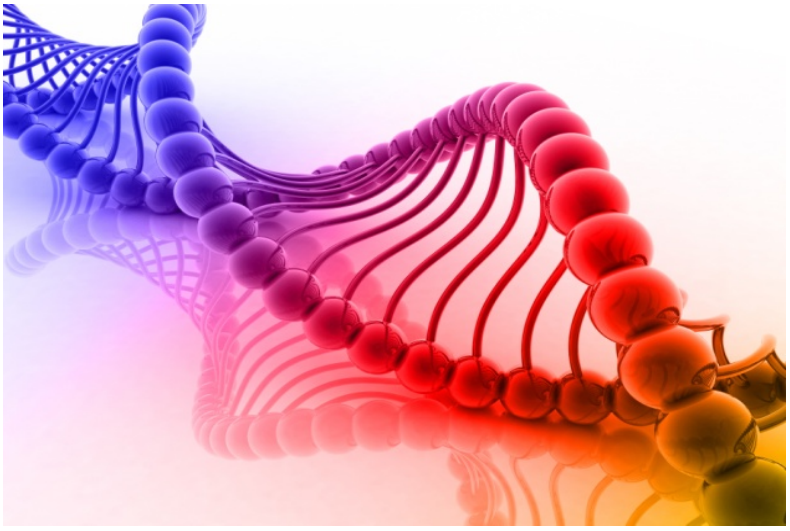

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COMMUNICATION

In vitro selection of a photo-responsive peptide aptamer using ribosome display†‡

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A photo-responsive peptide aptamer against microbeads immobilized streptavidin was isolated using *in vitro* selection combined with photo-manipulation. This is the first example of the introduction of a peptide aptamer in the photo-control of dynamic molecular recognition.

Artificial control of dynamic molecular recognition is an important and promising research area.[1] Light has been used as a dominant external stimulus to control various processes of dynamic molecular recognition.[2] For example, "photo-responsive crown ethers",[3] "a photo-chemically driven molecular machine"[4] and "a light-powered molecular pedal"[5] have been reported as supramolecular systems. A light-triggered switch has also been developed in dendritic polymers,[6a] material surfaces,[6b] catalyst systems,[6c] and bulk material structures.[6d] In addition, photo-responsive biopolymers (both DNA and peptide based) were also designed to photocontrol the interaction between biologically active molecules.[7] In all of the cases described above, a rigorous molecular design (commonly termed "rational design") was performed, by which a dynamic function induced by light was appended to the molecules. Recently several researchers reported peptides or oligonucleotide recognizing photo-responsive targets.[8] However, the design of the photo-responsive host molecule for arbitrary targets, which is structurally complicated or unknown, is difficult and was not achieved.

We have proposed an approach to achieve the photocontrol of dynamic molecular recognition with an arbitrary target by using an *in vitro* selection method. In this method, a random sequence oligomer library containing a photo-isomerizable molecule (*e.g.* azobenzene or spiropyran) is prepared and photo-responsive host molecules are selected against a target. We previously isolated photo-responsive hemin-binding RNA aptamers by *in vitro* selection and demonstrated that the aptamers exhibited photo-responsive peroxidase activity by forming a complex with hemin.[9] However, a peptide-based photoreactive aptamer has not been developed; although the greater diversity of polypeptide functional groups when compared with oligonucleotides may increase the possibility of higher affinity and specificity. In this communication, we isolated a photo-responsive peptide aptamer which recognizes a target by using ribosome display.

Phage display has been employed by many researchers in the selection of a peptide aptamer.[10] However, it is impossible to incorporate non-canonical amino acids into the display system. In order to incorporate non-canonical amino acids into the selection system, other selection systems using *in vitro* translation such as ribosome-display[11] or mRNA-display[12] have been developed.

Using the molecular level peptide display, Roberts and coworkers[13] selected biocytin containing peptides against streptavidin and a hybrid drug-peptide aptamer against *S. aureus* penicillin-binding protein PBP2a from a random sequence polypeptide library that was chemically modified after translation. Subsequently, it was recently reported that artificial amino acids carried by tRNA were incorporated into a peptide library using a cell-free protein synthesis system and peptide aptamers were selected from the library.[14] These methods enable the selection of peptide aptamers against arbitrary targets from a peptide library which contains non-canonical amino acids.

Here we used the incorporation of non-canonical amino acids in ribosome display[15] for the preparation of a photo-responsive peptide aptamer. tRNA carrying an amino acid coupled with an azobenzene residue was prepared in order to provide photo-responsiveness to the peptide aptamer. The selection protocol is schematically shown in Fig. 1 and explained in detail in the ESI.‡ First, *t*Boc-ε-(azobenzoyl)-lysine was synthesized and coupled with 5′-*O*-phosphoryl-2′-deoxycytidylyl-(3′-5′)adenosine (pdApC). Subsequently, the ε-(azobenzoyl)-lysine–pdApC was ligated with truncated tRNA carrying the amber anticodon. Similar to the random sequence library, an amber codon-containing random sequence library of DNA, $(NNN)_3TAG(NNN)_7$ and $(NNN)_7TAG(NNN)_3$ where N = G, C, T, or A, was prepared.

After *in vitro* transcription of the random sequence DNA library, a cell-free translation using the PURE SYSTEM in the

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‡ Electronic supplementary information (ESI) available: Synthesis of azobenzene-lysine; preparation of azobenzoyl-lysine-tRNA; *in vitro* selection; subcloning and sequencing; primer sequences; chemical synthesis of the peptide; binding assay. See DOI: 10.1039/c2cc36618e

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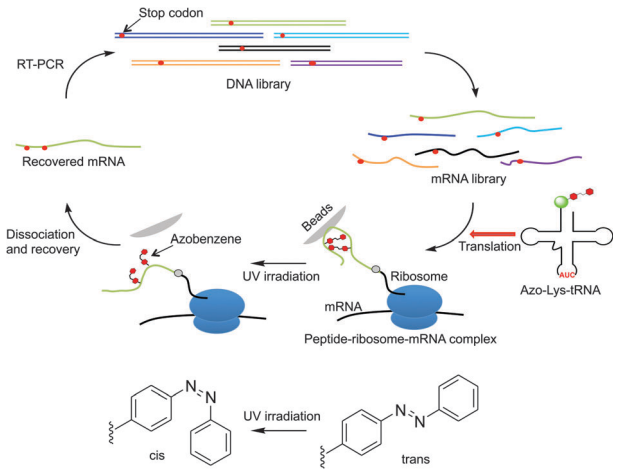

Fig. 1 The principle of *in vitro* selection using ribosome display and misacylated tRNA. A DNA library was transcribed into an mRNA library. The mRNA library and azo-Lys-tRNA (tRNA carrying azobenzene-coupled lysine) were then introduced into a cell-free translation system, resulting in the production of an azobenzene-modified peptide displayed on the surface of the ribosome. After affinity selection, the bound peptides were eluted by UV irradiation. The eluted complexes were collected and subsequently dissociated. The mRNA that encodes the peptide sequence was recovered and used for the next round of selection after RT-PCR.

presence of the tRNA carrying azobenzene-coupled lysine was performed to prepare the azobenzene-containing peptide library. The translation yield was about 10-fold lower due to the addition of the tRNA. The library forms a complex with each coding mRNA and ribosome by losing the stop codon and processing at low temperature, which is called the ribosome display system.[10] The library was incubated with magnetic microbeads immobilized with streptavidin for 30 min under dark conditions, in which almost azobenzene took *trans*-form, and the microbeads were washed with the same buffer. Subsequently, the beads were irradiated with ultraviolet light for 10 min. The irradiation of ultraviolet light was carried out by using a UV spot light instrument (from Hamamatsu Photonics) with a UV-D36B filter (from Asahi Technoglass). The intensity of the UV light was 6 mW cm^{-2}. In this step, peptides with *cis*-form azobenzenes can be released from beads, since conformational changes of the peptides by the *trans–cis* isomerization of azobenzenes are accompanied by the changes in affinity to streptavidin. Thus, the irradiated solution was recovered and the complex was dissociated by the addition of ethylenediaminetetraacetic acid. The recovered mRNA was collected and a reverse transcription (RT)-polymerase chain reaction (PCR) was performed for the amplification of DNA from the recovered mRNA. The DNA was employed for the next step of the selection process.

Table 1 Peptides synthesized by the solid phase method

Abbreviation	Sequence[a]
LA37	QA**VLIMVAVXASS**GQLGQFEGS<u>DYKDDDDK</u>
LA40	QA**HSCXVTIDVFF**GQLGQFEGS<u>DYKDDDDK</u>
LA81	QA**GVTXRRFIXYV**GQLGQFEGS<u>DYKDDDDK</u>
Scramble peptide	CYSCGSQVAFRLSLFGCSFGGD<u>YKDDDDK</u>

[a] Bold letters indicate peptide sequences selected from the library. Underlined letters indicate FLAG sequences. X indicates Lys residues coupled with azobenzene.

After five rounds of these selection processes, the selected sequence was cloned and the sequences were analyzed. The sequences are listed in Table S1, ESI.‡ Seventy-seven kinds of sequences were found from 81 clones. Two sequences VLIMVAVXASS (LA37, 48 and 91) and HSCXVTIDVFF (LA40, 43 and 93) were derived from three clones. In addition, there are five sequences containing two azobenzene-containing lysine residues. Here, two peptides derived from plural clones and one peptide containing two azobenzene groups were chosen, and these peptides were chemically synthesized by a solid phase method (see ESI‡). The former was considered to be major population of the selected peptides. The latter was considered to be more photo-responsive, because two photo-isomerizable moieties were present. Taking into consideration the low incorporation efficiency of non-canonical amino acids, the two incorporation sites-containing sequence was considered to be a high-binding candidate. In addition, the sequence was considered to be water-soluble because it had plural arginine residues which contribute to water-solubility.

Peptides containing the sequence of LA37, LA40 and LA81 with DYKDDDDKA (FLAG sequence) were synthesized (Table 1) and the binding behavior onto the microbeads was assayed by staining with a fluorescein isothiocyanate (FITC)-labeled anti-FLAG-antibody (Fig. 2). Higher binding affinity was observed for LA81 than found for LA37 or LA40, as shown in Fig. 2a. The dissociation constant (K_d) of the LA81 peptide bound to the microbead was calculated to be 6.31 μM by curve-fitting to a Langmuir isotherm. In addition, LA81 adsorbed onto the microbead under visible light irradiation but this adsorption was significantly reduced by UV irradiation (Fig. 2b). In contrast, a scrambled sequence peptide (control) did not bind and showed no photo-responsiveness.

The conformational change of LA81 in response to photo-irradiation was observed by ultraviolet (UV) and circular dichroism (CD) measurements (Fig. 3). The spectral change of LA81 (Fig. 3a) was similar to that of the monomer that is *t*Boc-ε-(azobenzoyl)-lysine (Fig. S1, ESI‡), whereas the efficacy of *trans–cis* isomerization is significantly different from each other. About 70% of *trans*-azobenzenes were isomerized to *cis*-form in the case of *t*Boc-ε-(azobenzoyl)-lysine, but only

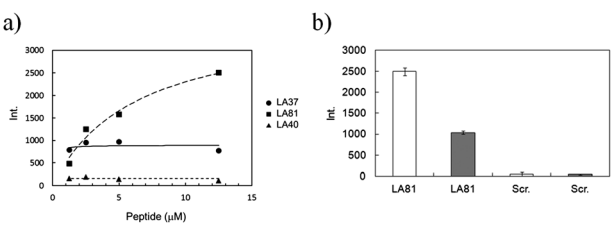

Fig. 2 Binding experiments on selected peptides. (a) Binding of peptides containing LA37, LA40 and LA81 sequences. The incubation was carried out at 20 °C for 1 h in TBS-T buffer (20 mM Tris, 137 mM NaCl, 0.1% Tween 20, pH 7.6). The bound microbead was stained with an FITC-labeled anti-FLAG-antibody and then quantified by a microplate reader. (b) Photo-responsive binding of peptides containing LA81 or a scrambled sequence. White bar: under the visible light irradiation conditions; grey bar: under the UV irradiation conditions. The intensity of the UV light was 6 mW cm^{-2}.

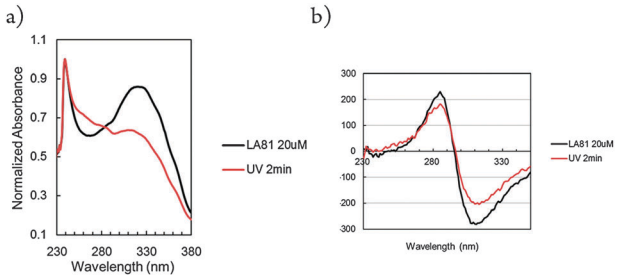

Fig. 3 (a) UV and (b) CD spectra of LA81 under irradiation of visible and ultraviolet light. In TBS-T buffer at room temperature.

30% in the case of LA81, suggesting that the isomerization of azobenzene in LA81 is more difficult than that in *t*Boc-ε-(azobenzoyl)-lysine due to the stereo-inhibition of adjacent amino acids. However, this result indicates that the *cis–trans* conformational change of azobenzene occurred in LA81. Although azobenzene itself has no CD signal, the LA81 peptide showed a CD signal between 250 and 350 nm, and UV irradiation altered the spectrum (Fig. 3b). The photoinduced change of the UV and CD spectra was reversible and could be repeated. The reason why photoswitching of azobenzene was not clearly 100% ON and OFF was considered to be due to the overlap of absorption of the *trans* and *cis* isomers in the 300–400 nm range. Complete ON–OFF switching will be achieved by using other photoresponsive molecules such as bridged azobenzene derivatives.[16]

This is the first example of the introduction of a peptide aptamer in the photo-control of dynamic molecular recognition. The application of the strategy proposed in this study renders *in vitro* selection of photo-responsive host molecules against various guests as promising. The photo-responsive peptide sequence will be available as a protein-tag for the purification of biologically significant protein. Moreover, the photo-responsive peptide that binds nanomaterials such as carbon nanotubes could also be applicable in particular photo-switching nanodevices.

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