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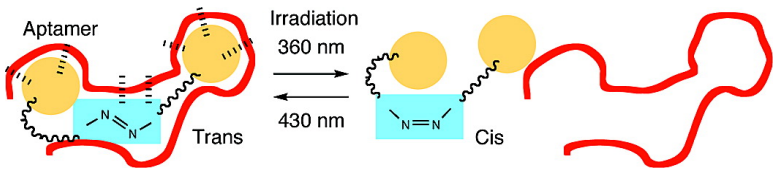

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Photoregulation of a Peptide−RNA Interaction on a Gold Surface

Gosuke Hayashi, Masaki Hagihara, Chikara Dohno, and Kazuhiko Nakatani*

Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research (ISIR), Osaka University, Ibaraki 567-0047, Japan

Received February 23, 2007; E-mail: nakatani@sanken.osaka-u.ac.jp

The discovery of riboswitching revealed novel paths for the regulation of gene expression by the binding of a small molecular ligand to RNA[1] and encouraged the study of molecular switches to modulate gene expression via the ligand−RNA interaction.[2] Toward this end, we need pairwise combinations of a ligand molecule and its target RNA in which interaction could be controlled, preferably by external stimuli.[3] While the molecular design of ligands targeting a particular RNA is still a difficult task, RNA aptamers that bind to the molecule of interest could be developed by an in vitro selection method.[4] We report here the combination of a photoresponsive peptide and its RNA aptamer pair as a molecular switching system, which could be turned on and off by the light-induced structural change of azobenzene incorporated into the peptide backbone.[5−7] The peptide, covalently immobilized on a gold surface, showed photoresponsive binding to the target RNA. The method reported here has the potential to provide a large number of ligand−RNA pairs that are useful for molecular switching systems.

The photoresponsive peptide **KRAzR** used in these studies consisted of three basic amino acids and a photochromic amino acid (Az)[8] containing the azobenzene chromophore (Figure 1). The RAzR motif was used with the expectations that (1) the guanidinium groups in the two arginines may bind to RNA not only through electrostatic interactions but also by hydrogen bonding to nucleotide bases, and (2) the Az amino acid inserted between the two arginines may produce a major effect from the structural change in azobenzene on the modulation of the spatial orientation of the two guanidinium groups. The N-terminal lysine was used to immobilize the peptide at the ϵ-amino group to the agarose gel in aptamer selection and the gold surface in the surface plasmon resonance (SPR) assay. Photoisomerization of **KRAzR** effectively proceeded at 360 nm from *E* to *Z* and at 430 nm from *Z* to *E*. The *Z*-isomer was thermally stable at room temperature in the dark for 10 h but gradually isomerized to the *E*-isomer under room light (Figure 2a). The *E*/*Z* ratio of the peptide was determined by the peak area in HPLC analysis with UV detection at the isosbestic point (282 nm) in the *E*−*Z* isomerization. Under room light, the peptide was a mixture of 75% *E*- and 25% *Z*-isomer (Figure 2b). Photoirradiation at 360 nm for 5 min led to the photostationary state, where the *E*/*Z* ratio was 5:95. Irradiation of the *Z*-predominant mixture at 430 nm for 5 min led to an *E*/*Z* ratio of 79:21.

The RNA aptamers binding to the peptide were obtained by in vitro selection from the 70 random nucleotides (N70) sequence pool of RNA (Figure 3a).[4] The DNA pools consisting of a forward primer sequence including T7 promoter and *EcoR* I restriction site, N70 random sequence, and a reverse primer sequence including the *BamH* I site were transcribed to RNA pools, which were applied to the agarose gel column of **KRAzR**. The RNA fractions retained on the peptide column were recovered by competitive elution with 6 mM **KRAzR**. After eight cycles of the selection, the sequence of aptamers binding to **KRAzR** was identified. No particular

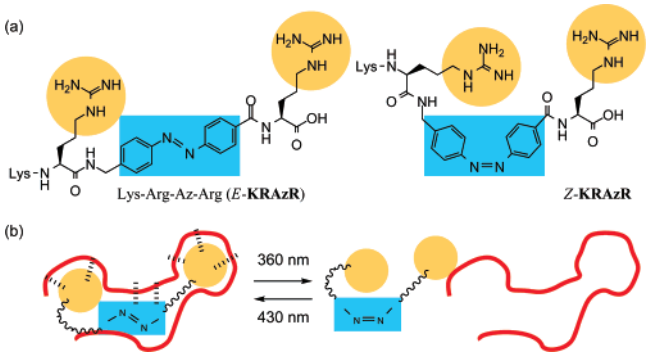

Figure 1. (a) Structures of the *E*- and *Z*-isomers of Lys−Arg−Az−Arg (**KRAzR**). Available special orientations for the two guanidinium groups (shown as the orange circles) were dependent on the configuration of azobenzene (shown in the blue rectangles). (b) Illustrations of photoregulation of **KRAzR** binding to its RNA aptamer (shown as a red line). The dashed lines signify hydrogen bonds to the guanidinium groups and hydrophobic interactions to the azobenzene chromophore.

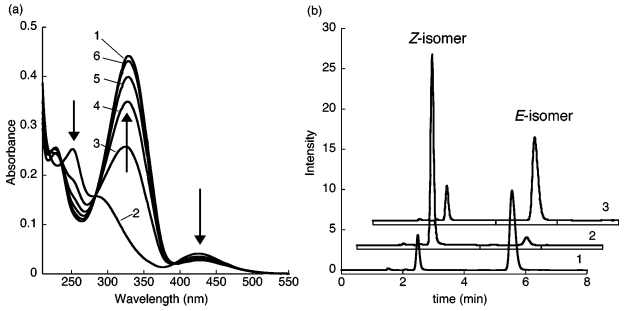

Figure 2. Photochromic properties of **KRAzR**. (a) Photoisomerization of *E* to *Z* by 360 nm irradiation followed by isomerization of *Z* to *E* under room light monitored by UV spectroscopy. Key: (1) **KRAzR** left under room light; (2−6) samples left under room light for 0, 15, 30, 45, and 60 min, respectively, after 360 nm irradiation for 5 min. (b) The *E*/*Z* ratio determined by HPLC profiles. Key: (1) **KRAzR** left under room light; (2) after 360 nm irradiation for 5 min; (3) subsequent irradiation at 430 nm for 5 min. HPLC detection was obtained at 282 nm.

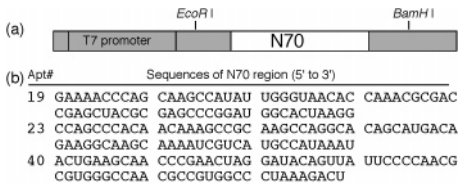

Figure 3. (a) The structure of the DNA pool used for in vitro selection. The gray areas are the sequence for hybridizing the forward (left) and reverse PCR primers. (b) The N70 sequence of RNA aptamers.

consensus sequences were found for 33 aptamers obtained by the selection (Table S1). The sequences of representative aptamers are shown in Figure 3b. The dissociation constants of isolated aptamers 19 (Apt19), 23 (Apt23), and 40 (Apt40) determined by SPR were 0.71, 2.4, and 1.8 μM, respectively. The K_d values of these aptamers

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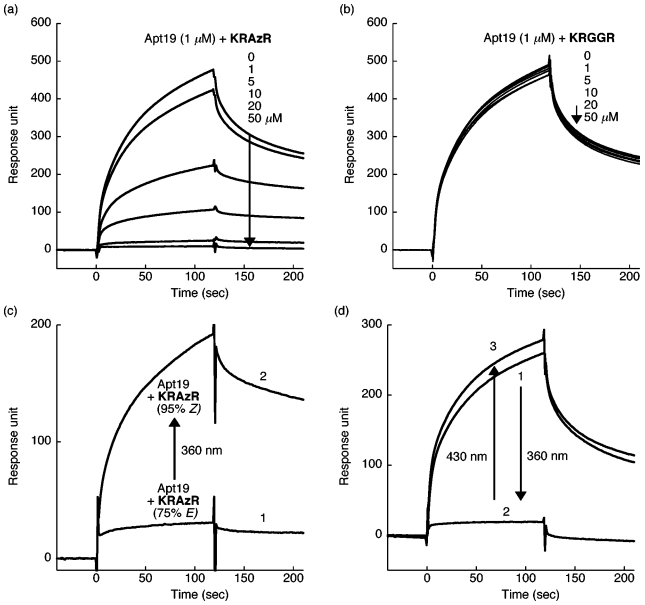

Figure 4. SPR assay for the binding of Apt19 to **KRAzR** immobilized on the gold surface. (a) Competitive binding assay of Apt19 (1 μM) with **KRAzR** (0, 1, 5, 10, 20, and 50 μM) in 50 mM Tris−HCl (pH 8.0), 250 mM NaCl, and 5 mM $MgCl_2$. (b) As for (a) except **KRGGR**. (c) Competitive binding assay of Apt19 (0.5 μM) with response to photoirradiation. Key: (1) Apt19 and **KRAzR** (75% *E*-isomer) (20 μM); (2) Apt19 with photoirradiated **KRAzR** (95% *Z*-isomer) at 360 nm for 5 min. (d) Effects of photoirradiation of **KRAzR** on the gold surface on the binding of Apt19. Key: (1) gold surface left under room light; (2) the surface after 360 nm irradiation; (3) subsequent irradiation of the surface at 430 nm.

are comparable to that of the reported aptamer for arginine, having a K_d of 0.33 μM.[4c]

We then focused our attention on the aptamer binding in response to the structural change upon photoisomerization. First, the structure−activity relationship of the peptide on the binding to Apt19 was investigated by competitive binding assay with the **KRAzR**-immobilized SPR sensor. Increasing the concentration of the peptide (0 to 50 μM) in the solution of Apt19 (1 μM) decreased the intensity of the SPR signal, indicating that the peptides on the gold surface and in the solution competitively bound to Apt19 (Figure 4a). Peptides having one to three glycines between two arginines (KRGR, KRGGR, and KRGGGR) did not compete with the immobilized **KRAzR** in the binding to Apt19 (Figure 4b). Both the Az amino acid and arginine did not bind to Apt19 competitively on the surface. In marked contrast to the *E*-isomer (75% *E*), the photoirradiated **KRAzR** at 360 nm for 5 min consisting of 95% *Z*-isomer could not competitively bind to Apt19 with the **KRAzR**-immobilized surface (Figure 4c). These experiments showed that the RAzR motif and the *E*-configuration of the azobenzene moiety were necessary for the binding of **KRAzR** to Apt19. The binding of **KRAzR** on the gold surface to Apt19 could be modulated by photoirradiation of the surface.[9]Upon photoirradiation of the **KRAzR**-immobilized surface at 360 nm for 5 min, the binding of Apt19 to the surface was decreased by more than 90% after 110 s of analysis (Figure 4d), whereas subsequent photoirradiation of the same surface with 430 nm light restored the aptamer binding to the surface. Photoregulation of the binding of **KRAzR** to Apt23 and Apt40 was also observed. Preliminary SPR imaging analyses with **KRAzR**-immobilized gold surface clearly showed that the association and dissociation of Apt19 to the surface is in fact fully reversible with response to the photoirradiation (Figure S8). These results indicate that the binding of **KRAzR** to its aptamers was fully responsive to the photoisomerization of the azobenzene chromophore and was controllable on the gold surface.

We here demonstrated that the in vitro selection toward a photoresponsive peptide effectively provided the ligand−RNA pair, in which the binding was controllable by photoirradiation both in solution and on the gold surface. A more sophisticated approach in terms of the design of the RNA pools and ligand molecules will provide molecular systems that are useful for controlling RNA functions by small molecular ligands with external stimuli.

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Supporting Information Available: Experimental procedures, photochemical properties of **KRAzR**, and the sequence aptamers. This material is available free of charge via the Internet at http://pubs.acs.org.

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