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Photoinduced growth system of peptide nanofibres addressed by DNA hybridization

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Spatiotemporal control of peptide nanofibre growth was achieved by photocleavage of a DNA-conjugated β-sheet forming peptide that is linked through a photoresponsive amino acid residue. Peptide nanofibres were selectively formed by photocleaving the conjugate on complementary DNA-immobilised glass substrate.

In nature, self-assembled structures consisting of peptides and proteins contribute to efficient molecular-level interactions, with virus capsids and microtubules being good examples.[1] The formation mechanism of natural supramolecular assemblies has been mimicked to design artificial peptide/protein assemblies.[2] Many α-helix coiled-coil and β-sheet structures have been employed as artificial self-assembling motifs, which are readily synthesised by the established methods.[3]

Stimuli-responsive peptides that change their conformations and self-assembling behaviours by external stimuli such as light, pH, redox reagents and metal cations have been reported.[4] Among these, photoresponsive peptides have the advantage of relatively short response time in changing their structures. Caged polar side chains could be used to change the polarity of side chains before/after UV irradiation, leading to changes in the secondary structure of the peptide attributed to electrostatic and hydrophobic interactions between peptides.[5] Photoresponsive amino acid residues were introduced in peptide sequences and used for cleavage[6,7] or formation[8] of the main chains by UV irradiation. Photoisomerization units were also introduced to reversibly control peptide conformations.[9]

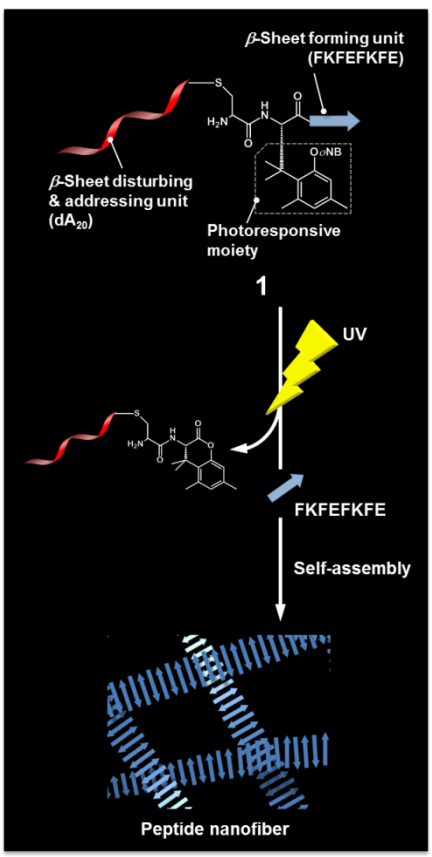

Fig. 1 Schematic illustration of the photocleavage reaction of DNA-conjugated peptide **1** and formation of peptide nanofibres.

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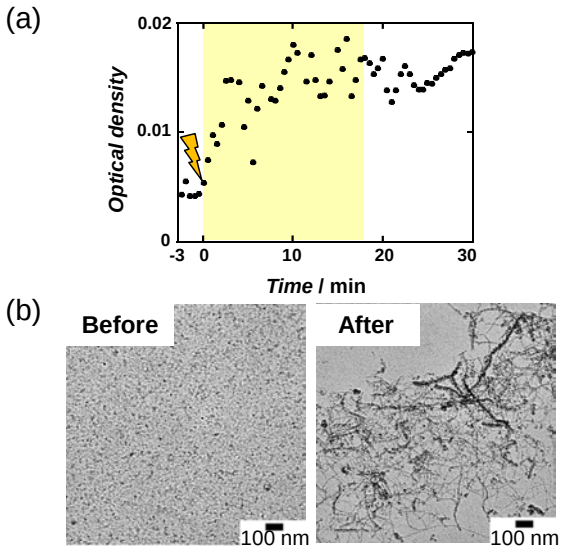

Fig. 2 (a) Time course of turbidity of a 10 μM solution of DNA-conjugated peptide **1** in water/acetonitrile (9/1, v/v) at 25°C. The starting point of UV irradiation is defined as 0 min. The irradiation time was a total of 12 min (20 s × 36) in the yellow region. (b) TEM images of **1** observed before/after UV irradiation. The samples were stained with 2% aqueous solution of sodium phosphotungstate.

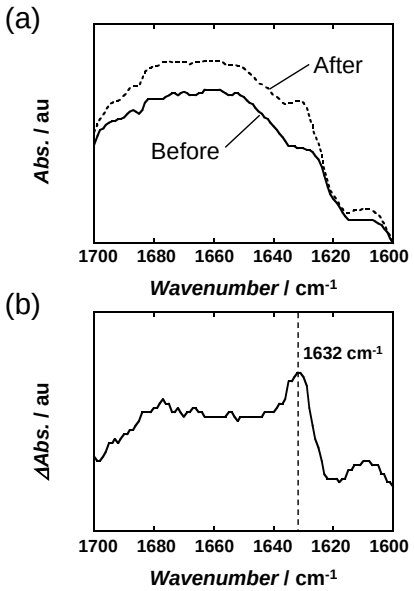

Fig. 3 (a) ATR–FT-IR spectra of DNA-conjugated peptide **1** before (solid) and after (dashed) UV irradiation. (b) The difference spectrum of the spectra in (a).

Photoresponsive peptides enable photocontrol of assemblies at a chosen timing. For example, Messersmith et al. showed a self-assembling system of a β-sheet forming peptide triggered by near-infrared light exposure to liposomes consisting of $CaCl_2$ and bacteriochlorophyll.[10] On the other hand, Stupp et al. reported that UV irradiation induced fibre formation using peptide amphiphiles with a photoacid generator in liposomes.[11] However, to the best of our knowledge, there are no examples of photoresponsive peptides that address themselves to an arbitrary place for fibre formation.

Herein, we report spatiotemporal control of peptide nanofibre growth by photocleavage of a DNA-conjugated peptide **1** (Fig. 1). The 8-mer peptide FKFEFKFE[13] was designed as a β-sheet forming unit to construct the peptide. Single-strand DNA (dA_{20}) was employed as the β-sheet disturbing unit because of its electrostatic repulsion. In addition, we expected that conjugate **1** is addressed on complementary dT_{20}-immobilised glass substrate by its hybridization.[12] To link these two conflicting units, a photoresponsive amino acid ((*S*)-3,3-dimethyl-3-[2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl]-2-(9-fluorenylmethoxycarbonylamino) propionic acid) residue developed by Shigenaga et al. was employed.[6a,7,14] Deprotection of the *ortho*-nitrobenzyl (*o*NB) group by UV irradiation followed by nucleophilic attack of the phenoxide anion leads to cleavage of the peptide bond by an intramolecular cyclisation reaction. The resulting free FKFEFKFE peptide will self-assemble into peptide nanofibres (Fig. 1).

The dA_{20} bearing an amino group via a hexamethylene chain at the 5′ end was reacted with the activated ester of a heterofunctional linker, *N*-(4-maleimidobutyryloxy)sulfosuccinimide sodium salt, to introduce a maleimide group to the DNA. The peptide CXFKFEFKFE (X denotes the photoresponsive amino acid) was synthesised by Fmoc solid-phase peptide synthesis in 39% yield, purified by reverse phase high performance liquid chromatography (RP-HPLC) and confirmed by electrospray ionisation mass spectrometry (ESI–MS).[15] The DNA-conjugated peptide **1** was synthesised by reaction of the thiol group of the peptide with the maleimide group of dA_{20} in 9% yield, purified by RP-HPLC, and confirmed by matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry (MALDI-TOF-MS).

The photocleavage reaction of the DNA-conjugated peptide **1** was traced by RP-HPLC analysis (Fig. S1 in ESI). After UV irradiation (365 nm, 4 W/cm^2) onto a 1 μM aqueous solution of **1**, the peak of the DNA-conjugated peptide **1** almost disappeared. Two new peaks appeared which were assigned to the DNA fragment and FKFEFKFE peptide, respectively.[16]

As shown in Fig. 2a, turbidity (optical density at 400 nm) of a 10 μM solution of **1** in water/acetonitrile (9/1, v/v) changed dramatically within 10 min during UV irradiation. Before UV irradiation, **1** was dispersed because of the electrostatic repulsion between the DNA moieties. An abrupt increase in turbidity with UV irradiation indicates that the FKFEFKFE peptides released by photocleavage of **1** formed assemblies. Transmission electron microscopy (TEM) observation showed only spherical structures with a size of ca. 10 nm before UV irradiation; whereas, fibrous assemblies 10–20 nm in width were observed after irradiation (Fig. 2b). These results indicate that FKFEFKFE peptide was generated by UV irradiation to self-assemble into nanofibres. The secondary structure of self-assembled peptides was studied using attenuated total reflection/Fourier transform infrared (ATR-FT-IR) spectra before/after UV irradiation (Fig. 3). It is noteworthy that the difference spectrum showed a characteristic peak at 1632 cm^{-1}, indicating formation of β-sheet structures.[17]

To demonstrate the addressing ability of conjugate **1**, dT_{20}- and dA_{20}-immobilised glass substrates were prepared according to the literature (Fig. S2).[18] Hybridization of **1** on the dT_{20}-immobilised glass substrate was performed using a 10 μM solution of **1** in water/acetonitrile (9/1, v/v), which was confirmed by fluorescence microscopic observation using double-strand DNA-selective fluorescence dye, 4′,6-diamidino-2-phenylindole (DAPI, Fig. S3). The peptide density at the hybridized surface was estimated as ca. 0.15 peptide/nm^2,[18] which would be sufficient for the liberated peptides to form peptide fibrous structures (see ESI). After washing and subsequent UV irradiation, the surface of the substrate was observed using scanning electron microscopy (SEM). Micrometre-

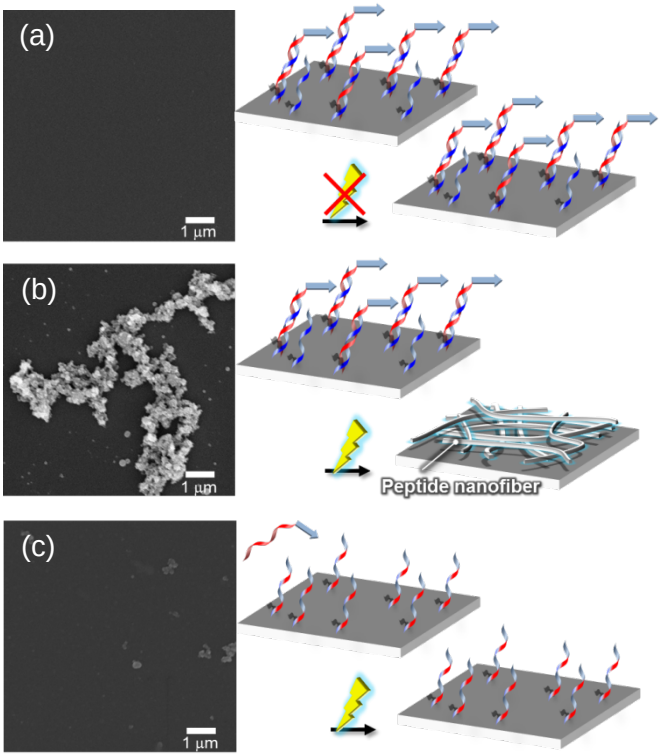

Fig. 4 SEM images of microstructures formed by photocleavage of **1** on dT_{20}-immobilised glass substrates: (a) before UV irradiation, (b) after UV irradiation, (c) SEM image of **1** on dA_{20}-immobilised glass after UV irradiation. SEM samples were coated with Pt–Pd.

sized fibril structures were observed on the dT_{20}-immobilised glass substrate after UV irradiation (Fig. 4b), whereas such structures were barely observed before irradiation (Fig. 4a). These data show that the self-assembly of peptide nanofibres is triggered by UV irradiation. Formation of the local self-assembling peptide nanofibres could be explained by assuming that some of the photogenerated FKFEFKFE peptides accidentally become the starting points of nanofibre growth. In contrast, only small amounts of microstructures of **1** were observed on dA_{20}-immobilised glass substrate even after UV irradiation (Fig. 4c).

In conclusion, we have demonstrated that a photocleavage reaction of a DNA-conjugated peptide proceeded by UV irradiation and the released β-sheet-forming peptides form nanofibres. Disturbing the self-assembly of the conjugates before UV irradiation and guiding them to the immobilised complementary DNAs was successfully accomplished using the DNA moiety. Peptide nanofibres were selectively formed by photocleaving the conjugate on complementary DNA-immobilised glass substrate. The present system could be integrated into other applications, such as a highly sensitive DNA sensing device.

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