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A distance-controlled oligopeptide linker as a novel photo-induced energy transfer switch by secondary structural transition

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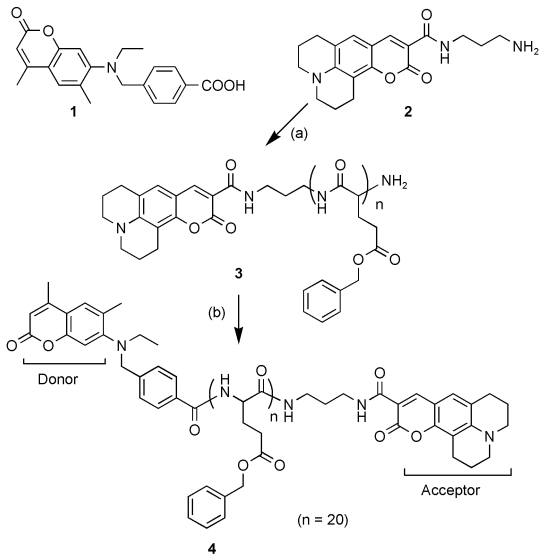
Received (in Cambridge, UK) 3rd January 2003, Accepted 11th February 2003
First published as an Advance Article on the web 25th February 2003

By using an oligopeptide chain as a functional linker and introducing coumarin 2 and coumarin 343 at the chain ends, an effective photo-induced energy transfer system was constructed and energy transfer from coumarin 2 to coumarin 343 was switched on and off by a solvent-induced helix–coil secondary transition of the oligopeptide chain.

Photo-induced energy transfer systems that are capable of harvesting light and transferring or transforming absorbed energy have attracted great interest in recent years because of their applicability in such fields as light emitting diodes or photonic devices. For this reason, a wide variety of organic,[1] organometallic,[2] supramolecular,[3] polymeric,[4] and dendritic[5] systems have been developed. Energy transfer between fluorescent probes has also been used as a powerful tool for structural analysis of DNAs and proteins.[6] Efficient photo-induced electron/energy transfer through a DNA duplex is the subject of current interest.[7] Biomacromolecules like DNAs and polypeptides are prone to have rigid and highly ordered structures due to intramolecular hydrogen bonds and, therefore, are suitable for use as linkers in energy transfer systems. Several groups have previously reported energy transfer systems containing chromophores along oligopeptides.[8] However, few have reported highly efficient, switchable, and distance controlled systems by making the best use of the oligopeptide properties. Here, we report a simple and facile method for constructing efficient and distance-controlled energy transfer systems by introducing chromophores at each end of the oligopeptide linker. The distance between the chromophores can easily be tuned by controlled ring-opening polymerization of amino acid *N*-carboxyanhydride (NCA), and energy transfer between the chromophores through the oligopeptide is found to be switched on and off by a solvent-induced helix–coil transition of the oligopeptide linker.

Chromophores coumarin 2 and coumarin 343 were used as a donor and an acceptor set, respectively, as recently employed by Fréchet and coworkers.[9] This set meets the requirement for photo-induced energy transfer that the donor emission band has a large overlap with the acceptor absorption band. In addition, coumarin 2 displays a large Stokes' shift, which lowers the probability of self-quenching. Acceptor **2** bearing an aminopropyl group was used as an initiator for ring-opening polymerization of γ-benzyl-L-glutamate NCA, and the N-terminal of the resultant poly(γ-benzyl-L-glutamate) (PBLG) (**3**) was capped by donor **1** which bears a benzoic acid moiety.† Thus, PBLG (**4**) having a set of donor and acceptor chromophores at the N- and C-terminals of the chain, respectively, was obtained (Scheme 1). The number-average degree of polymerization can be controlled simply by adjusting the NCA/initiator molar ratio when primary amines are used as the initiator.[10] In this study, this was set at 20, and the average molecular weight of the peptide **3** was confirmed to be 4710 by GPC with a single and sharp molecular weight distribution.‡ To ensure the absence of low molecular weight components, the obtained PBLG (**4**) was also purified by GPC. PBLG is known to have stable α-helical rigid conformation in most common solvents,[11] and the average distance between the donor and the acceptor is about 3 nm when PBLG (n = 20) takes the α-helix conformation.



Scheme 1 Synthesis of donor–$PBLG_{20}$–acceptor model. (a) γ-BLG–NCA, DMF, 96%. (b) **1**, EDC, DMAP, CH_2Cl_2, 92%.

The absorption spectrum of **4** in CH_2Cl_2 shows two absorption maxima at 345 and 420 nm, which correspond to those of the model donor **1** and acceptor **2**, respectively (Fig. 1(a)). This result indicates that **4** contains both the donor and the acceptor units at the PBLG chain ends. Note that **3** has little or almost negligible absorption around 345 nm, which confirms that it is possible to excite the donor unit of **4** selectively at this wavelength.

The secondary structure of **4** was examined by means of circular dichroism (CD) and infrared spectroscopy. In the CD spectrum of **4** in CH_2Cl_2, a negative Cotton effect was observed around 230 nm (glutamate unit), indicating that **4** forms a right-handed α-helix (Fig. 1(b)). This fact was further confirmed by the IR spectrum of the cast film of **4** from CH_2Cl_2, which showed amide I and II bands at 1651 and 1548 cm^{-1}, respectively, which is typical for α-helical conformation.[11] On the other hand, a cast film of **4** from DMSO, which is known as a helix-breaking solvent,[12] showed blue-shifted amide I and red-shifted amide II bands at 1674 and 1510 cm^{-1}, respectively,

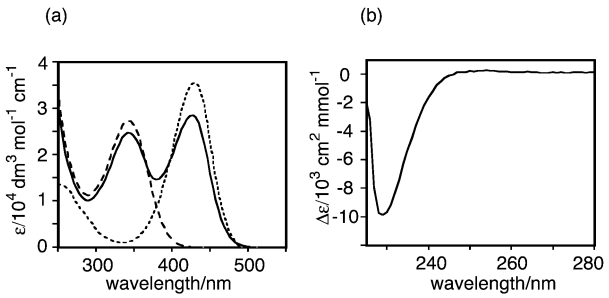


Fig. 1 (a) Absorption spectra of **1** (– – –), **3** (···) and **4** (—) in CH_2Cl_2, (b) CD spectrum of **4** in CH_2Cl_2.

DOI: 10.1039/b212851a

indicating that PBLG was in a random coil state caused by the destruction of intramolecular hydrogen bonds.

Fig. 2(a) illustrates the fluorescence spectra of **1** and **4** in both CH_2Cl_2 and DMSO by selective excitation of the donor unit at 345 nm. In CH_2Cl_2, the donor **1** shows strong blue fluorescence at 420 nm, but the acceptor **3** shows practically no fluorescence around 460 nm. In the case of **4**, however, fluorescence from the donor unit at 420 nm was considerably quenched and emission from the acceptor unit at 460 nm appeared, indicating that efficient energy transfer took place from the donor unit to the acceptor unit. The observed fluorescent properties of **4** were not affected by the concentration over the range of 10^{-5}–10^{-7} mol dm^{-3}, excluding the possible contribution of intermolecular energy transfer processes. The energy transfer efficiency was calculated to be 91% from the degree of quenching of the donor emission, which is high enough for the donor–acceptor distance of 3 nm.[13] Similarly, acceptor fluorescence and quenching of the donor fluorescence were observed in DMF, a highly polar solvent. However, only the donor fluorescence at 420 nm was observed without any sign of quenching in DMSO, indicating that energy transfer did not take place at all in DMSO. In the CH_2Cl_2/DMSO mixture, increasing the DMSO content resulted in a decrease of the energy transfer efficiency, and no energy transfer occurred above 80 vol% of DMSO. Decreasing the DMSO content to less than 10 vol% by dilution with CH_2Cl_2 induced fluorescence of the acceptor with concomitant quenching of the donor fluorescence again, showing that the effect of DMSO is reversible. The effect of DMSO on the energy transfer is in sharp contrast to that of similarly polar DMF (Table 1). In CH_2Cl_2, PBLG takes rigid α-helical conformation suitable for energy transfer, allowing efficient energy transfer (91%) over 3 nm. It is known that DMF is not effective to induce secondary transition of PBLG into flexible random coil but DMSO can effectively induce this transition.[14] Therefore, observed switching off of the energy transfer process by DMSO is attributed to the helix to random coil transition of the PBLG linker (Fig. 2(b)). Since Förster length R_0,[15] which is the distance when energy transfer occurs with 50 % efficiency, is estimated to be 2.53 nm in this sets, energy transfer efficiency is below 1% when average inter-chromophore distance is over 6 nm. Therefore, it is likely that the donor and acceptor units in **4** are separated more than 6 nm in the random coil state.

In summary, we developed a simple method to construct a set of chromophores separated by a distance-controlled oligopeptide linker, and demonstrated that the system serves as an efficient photo-induced energy transfer system which can be switched on and off by a helix–coil secondary transition of the oligopeptide linker. These results offer a facile method for developing energy transfer systems with various peptide linkers.

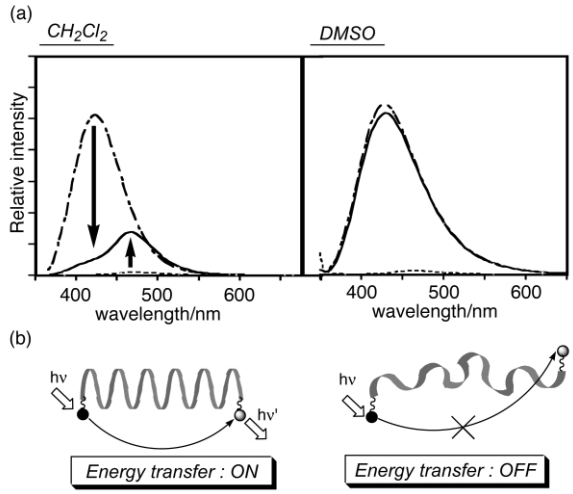


Fig. 2 (a) Fluorescence spectra of **1** (— -), **3** (– – –) and **4** (—) in CH_2Cl_2 and DMSO, (b) schematic illustration of the secondary structure of **4** in both CH_2Cl_2 and DMSO.

Table 1 Energy transfer efficiency of **4**

Solvent (CH_2Cl_2/DMSO)	Structure of **4**	Energy transfer efficiency (%)
100/0	α-helix	91
80/20	mixture	84
60/40		55
40/60		18
20/80	random coil	0
0/100		0
0/100 (DMF)	α-helix	82

We are grateful to Prof. Takashi Kato (Department of Chemistry and Biotechnology, Faculty of Engineering, Univ. of Tokyo) for helping with the GPC measurement.

Notes and references

† Compounds **1** and **2** were prepared as follows: reaction of coumarin 2 and methyl 4-(bromomethyl)benzoate following hydrolysis yielded **1** (25%), high-resolution MS (FAB): *m*/*z* calcd: 352.1471; found: 352.1556. Coumarin 343 was reacted with *N*-(3-aminopropyl) carbamic acid *tert*-butyl ester, which following deprotection of the Boc group afforded **2** (64%), high-resolution MS (FAB): *m*/*z* calcd: 342.1739; found: 342.1818.

‡ GPC analysis was performed at 25 °C with a TSKgel GMH_{HR}-N column (300 × 7.8 mm), DMF as eluent, and narrow-polydispersity poly(styrene) standards as a reference. The calculated molecular weight of the 20mer of peptide **3** is 4727, which is in agreement with the observed value (4710).

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