Sequential multistep energy transfer: enhancement of efficiency of long-range fluorescence resonance energy transfer

Shun-ichi Kawahara, Tadafumi Uchimaru* and Shigeo Murata

Department of Physical Chemistry, National Institute of Materials and Chemical Research, Agency of Industrial Science and Technology, MITI, Tsukuba Science City 305-8565, Japan. E-mail: t_uchimaru@home.nimc.go.jp

Received (in Cambridge, UK) 14th December 1999, Accepted 15th February 1999

The efficiency of long-range (*ca.* 80 Å) fluorescence energy transfer was enhanced about 1.5 times by a third chromophore located midway between two chromophores, which suggests sequential multistep energy transfer across the three chromophores.

Fluorescence resonance energy transfer (FRET)[1] is widely used to estimate chromophore separation and structure on a nanometre scale.[2] FRET in a system consisting of a fluorescent energy donor and acceptor has been extensively studied.[1b] The FRET technique has been frequently applied to probe biological and other complex systems.[3]

Recently, covalently linked porphyrin arrays have attracted much attention as biomimetic models.[4] This is because multiporphyrin assemblies are involved in the reaction centre of photosynthesis[5] and light-harvesting antenna complexes.[4] Much effort has been directed towards the development of such model systems. Covalently linked chromophore arrays will offer new opportunities for exploring long-range energy transfer and also electron transfer systems. A few reports of non-covalently linked chromophore arrays have also been described.[6]

Here we report a non-covalently linked chromophore array indicating sequential FRET across three different chromophores. We have introduced different chromophores in a duplex of oligodeoxyribonucleic acid (oligo-DNA) in a sequential manner. We have revealed that the efficiency of FRET across the two chromophores at both ends of the oligo-DNA duplex is enhanced by a third chromophore located midway between them.

6-Carboxyfluorescein (**1**), 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (**2**) and 6-carboxy-X-rhodamine (**3**) shown in Fig. 1(*a*), were used as chromophores. These chromophores were introduced at the 5′-terminal position of oligo-DNA 25-, 15-, and 10mers through an aminohexyl phosphate linker: the carboxy groups located at the 3-position of the chromophores were connected *via* amide linkages with the terminal amino group of the linker. The sequence of the oligo-DNA 15- and 10mers are complementary to that of the 25mer. Thus, these oligomers form typical B type duplexes. As shown in Fig. 2, we

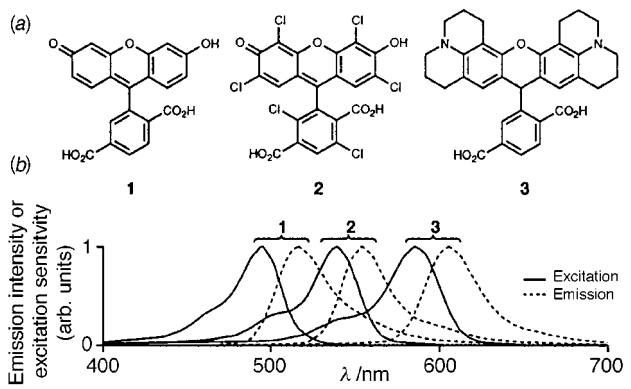

Fig. 1 (*a*) Structures of chromophores. (*b*) Excitation and emission spectra of each choromophore normalized at maximum.

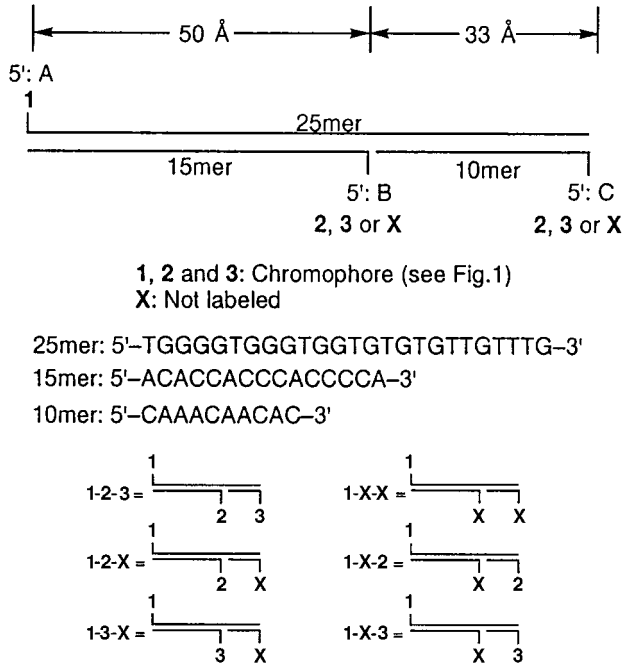

Fig. 2 DNA sequences and chromophore introduced positions.

call the 5′-terminal positions of the 25-, 15- and 10mers positions A, B, and C, respectively. Chromophore **1** was introduced at position A, while chromophores **2** and **3** were introduced at position B or C. Owing to characteristic structural features of the B type duplex, the chromophores introduced at position A, B, and C are located on the same side of the duplex chain. The distances between positions A and B and B and C are about 50 and 33 Å, respectively. Thus, position C is located *ca.* 80 Å from position A (see Fig. 2).

Fig. 1(*b*) shows the normalized emission and excitation spectra of the chromophores. The emission spectra of six duplexes (**1**-**2**-**3** through **1**-**X**-**X**,† see Fig. 2) excited at 450 nm are shown in Fig. 3(*a*) and (*b*).‡ The 450 nm light is absorbed by chromophore **1**, while the absorptions of chromophores **2** and **3** are quite weak or completely negligible at this wavelength [see Fig. 1(*b*)]. FRET from chromophore **1** to **2** or **3** was evidenced by fluorescence emission from chromophore **2** or **3**. The intensity of fluorescence from chromophore **1** was reduced as a

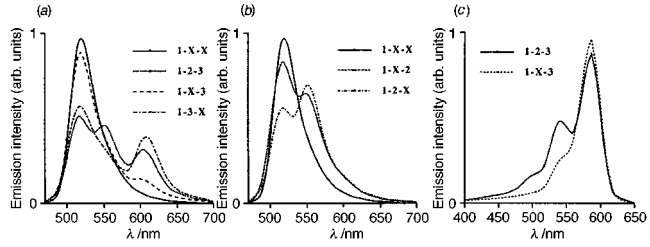

Fig. 3 (*a*) and (*b*) Emission spectra of each duplex. (*c*) Excitation spectra of **1**-**2**-**3** and **1**-**X**-**3**.

consequence of FRET across the chromophores. Correspondingly, fluorescence emission from chromophores **2** and **3** was observed. Table 1 shows the fluorescence quantum yields of each chromophore in the duplex relative to those of the same chromophore measured under the conditions without FRET.§

The results in Table 1 show that the efficiency of energy transfer from **1** to **2** or **3** decreases with the increase in the separation between the chromophores: the efficiency of energy transfer between position A and C with larger separation was lower than that between position A and B. Φ_f^{rel}(**2**) was reduced from 54% in duplex **1-2-X** to 32% in **1-X-2**. Conversely, for chromophore **1**, Φ_f^{rel}(**1**) was smaller in duplex **1-2-X** (57%) than in duplex **1-X-2** (85%). The same trend was seen for energy transfer from chromophore **1** to **3**. However, the efficiency of energy transfer from chromophore **1** to **3** was found to be lower than that from **1** to **2**. This is due to the fact that the fluorescence–absorption overlap between chromophores **1** and **3** is smaller than that between chromophores **1** and **2** [see Fig. 1(*b*)]. Φ_f^{rel}(**3**) in duplex **1-3-X** and **1-X-3** was 36 and 10%, respectively. Φ_f^{rel}(**1**) was 58 and 90%, respectively. These results indicate that our non-covalently linked chromophore arrays show normal behavior regarding energy transfer across the two chromophores.

Meanwhile, Φ_f^{rel}(**3**) was increased from 10% in **1-X-3** to 16% in **1-2-3** (see Table 1). Chromophore **2** inserted at the midway position B enhances the efficiency of the energy transfer from chromophore **1** to **3** located at positions A and C, respectively. Fluorescence excitation spectra of duplexes **1-2-3** and **1-X-3** observed at 605 nm (fluorescence maximum of chromophore **3**) are shown in Fig. 3(*c*). The excitation spectra suggested that the absolute fluorescence quantum yield of chromophore **3** was almost the same in these duplexes. Thus, we can safely conclude that the efficiency of FRET from chromophore **1** to **3** between position A and C was increased about 1.5 times due to chromophore **2** being inserted at midway position B.

Enhancement of the efficiency of energy transfer from chromophore **1** to **3** in duplex **1-2-3** can be interpreted as a consequence of sequential two-step energy transfer: energy transfer from chromophore **1** to **2** and subsequent energy transfer from chromophore **2** to **3**. Namely, chromophore **2** should work as a relay station for energy transfer, which suggests that use of an additional chromophore as a relay station should lengthen the range of energy transfer. Furthermore, sequential energy transfer *via* more than two relay stations may be possible. Such energy transfer might be called sequential multistep energy transfer. Sequential multistep energy transfer will provide us with information about the distance between chromophores with a separation larger than 100 Å, which is difficult using a simple energy transfer system consisting of just a fluorescent donor and acceptor. Thus, sequential energy transfer would be useful for research in various fields, not only in the fields of biochemistry[3] but also in the fields of supramolecular chemistry.[7]

In summary, we introduced three different chromophores in a duplex of oligo-DNA in a sequential manner. The efficiency of FRET across two chromophores was shown to be increased by a third chromophore located at the midway position between them. The third chromophore works as a relay station in energy transfer.

We thank Professor K. Tokumaru of the University of the Air for helpful discussions, and Science and Technology Agency for financial support.

Table 1 Values of Φ_f^{rel}(***n***)

Sequence	**1**	**2**	**3**
1-2-3	0.51	0.29	0.16
1-2-X	0.57	0.54	—
1-X-2	0.85	0.32	—
1-3-X	0.58	—	0.36
1-X-3	0.90	—	0.10

Notes and references

† **1-2-3** represents the oligo-DNA duplex possessing chromophores **1**, **2** and **3** at positions A, B and C, respectively. **X** means that no fluorescent chromophore is introduced at the position(s).

‡ All the fluorescence spectra were measured under the following conditions: 1 mM phosphate buffer (pH 7.0) with 0.1 M NaCl and 0.1 mM EDTA to remove the effect of contaminating metals. T = 20 °C. Concentration of oligomers = 1.0 μM. Under these conditions, three oligomers form duplexes (T_m^1 = 36–38 °C for dissociation of 10mer, T_m^2 = 60–63 °C for 15mer).

§ We derived the relative fluorescence yield, Φ_f^{apr}(***n***) in each duplex on the basis of observed fluorescence intensities of the chromophores. We employed eqn. (1), where ***n*** = **1**, **2** or **3**, Em(***n***)^{obs} = observed fluorescence

$$\Phi_f^{apr}(\boldsymbol{n}) = \left(\frac{\mathrm{Em}(\boldsymbol{n})^{obs}}{\mathrm{Em}(\boldsymbol{n})^{max}}\right)\left(\frac{\mathrm{EC}(\boldsymbol{n})}{\mathrm{EC}_{450}(\mathbf{1})}\right) \quad (1)$$

intensity of ***n*** in the FRET system excited at 450 nm, Em(***n***)^{max} = observed fluorescence intensity of ***n*** excited at the absorption maximum, EC(***n***) = extinction coefficient of **1** (48000/495 nm), **2** (79700/540 nm) and **3** (91200 /585 nm) under the present experimental conditions, and EC_{450}(**1**) = extinction coefficient of **1** at 450 nm (10800), calculated from excitation intensity and extinction coefficient at 495 nm.

However, the absorption of chromophore **2** at 450 nm cannot be completely neglected. Fluorescence emission from chromophore **2** results from not only energy transfer from **1** but also partially by direct excitation of **2** by irradiation at 450 nm. In duplex **1-2-3**, energy transfer will occur from directly excited **2** to **3**, which partially contributes to Φ_f^{apr}(**3**). We deducted these contributions of direct excitation of **2** from Φ_f^{apr}(**2**) and Φ_f^{apr}(**3**) and then derived the relative fluorescence yields of these two chromophores. Hereafter, we refer to these quantities as the relative fluorescence yields Φ_f^{rel}(***n***). The values of Φ_f^{rel}(***n***) are the fluorescence yields of emission from each chromophore solely due to the contribution of the energy transfer from chromophore **1**. For chromophore **1**, Φ_f^{apr}(**1**) = Φ_f^{rel}(**1**). In addition, for duplexes **1-3-X** and **1-X-3**, which do not possess chromophore **2**, Φ_f^{apr}(**3**) = Φ_f^{rel}(**3**). Thus, the relative fluorescence yields Φ_f^{rel}(***n***) in Table 1 do not include the contribution of fluorescence emission due to direct excitation of chromophore **2**.

1 (*a*) T. Förster, *Ann. Phys.*, 1948, **2**, 55; (*b*) B. Wieb Van Der Meer, G. Coker, III and S.-Y. S. Chen, *Resonance Energy Transfer Theory and Data*, VCH Publishers, New York, 1994.

2 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.

3 For example, A. I. H. Murche, R. M. Clegg, E. Kitszing, D. R. Dukett and D. M. J. Lilley, *Nature*, 1989, **341**, 763; T. Tuschl, C. Gohlke, T. M. Jovin, E. Westhof and F. Eckstein, *Science*, 1994, **266**, 785; R. C. Gupta, E. I. Golub, W. S. Marc and R. M. Charles, *Proc. Natl. Acad. Sci. USA.*, 1998, **95**, 9843.

4 D. L. Officer, A. K. Burrell and D. C. Reid, *Chem. Commun.*, 1996, 1657; A. Osuka and H. Shimizu, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 135; J.-P. Stachan, S. Gentemann, J. Seth, W. A. Kalsbech, J. S. Lindsey, D. Holten and D. F. Bocian, *J. Am. Chem. Soc.*, 1997, **119**, 11191.

5 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawethornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell and N. W. Isaacs, *Nature*, 1995, **374**, 517; S. Karrasch, P. A. Bullough and R. Ghosh, *EMBO J.*, 1995, **14**, 631.

6 P. Tecilla, R. P. Dixon, G. Slobodkin, D. S. Alavi, D. H. Waldeck and A. D. Hamilton, *J. Am. Chem. Soc.*, 1990, **112**, 9408; C. M. White, M. F. Gonzalez, D. A. Bardwell, L. H. Rees, J. C. Jeffery, M. D. Ward, N. Armaroli, G. Calogero and F. Barigelletti, *J. Chem. Soc., Dalton Trans.*, 1997, 727.

7 F. Vögtle, *Supermolecular Chemistry*, Maruzen, Tokyo, Wiley, West Sussex, 1995.

Communication 8/09728C