J|A|C|S COMMUNICATIONS

Published on Web 10/21/2009

DNA-Based Supramolecular Artificial Light Harvesting Complexes

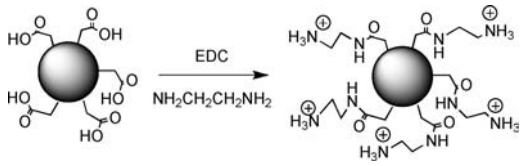
Challa V. Kumar* and Michael. R. Duff, Jr.

Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269-3060

Received June 4, 2009; E-mail: Challa.Kumar@uconn.edu

Successful design and synthesis of inexpensive, high efficiency, robust, artificial light harvesting complexes are essential for efficient collection, capture, and conversion of solar energy, which is a major renewable form of green energy.[1] Solar energy is distributed over a wide range of wavelengths, received on this planet at low energy densities, and efficient harvesting of solar energy continues to be a grand challenge. Solar-derived energy continues to be expensive, and efficient light harvesting technologies have a high potential to reduce the cost and make the technology economically viable. Engineered DNA−protein complexes are used here as novel supramolecular scaffolds to construct robust, inexpensive, artificial light harvesting complexes via self-assembly. These systems are novel, have a significant potential for self-repair, and complement existing technologies based on covalently linked donor−acceptor chromophores[2,3] or organized assemblies.[4−12] The very first examples of light harvesting units based on DNA−protein complexes are reported here, and the DNA−protein complexes organize, orient, and retain the necessary molecular components at specific sites for improved collection of light energy.

Scheme 1. Activation of the Carboxyl Groups of BSA (Gray Sphere) and Amidation with Ethylenediamine To Produce the Corresponding Cationized BSA



Our strategy is to chemically modify a suitable protein host to create artificial DNA−protein complexes and self-assemble donor and acceptor chromophores at specific locations within these superstructures. For example, a large number of cationic dyes are known to bind to DNA,[13] and we pioneered the design of a number of fluorescent probes which bind to proteins avidly.[14,15] These components spontaneously self-assemble to form donor−acceptor−DNA−protein complexes, and they facilitate singlet−singlet energy transfer from the DNA-bound donor to the protein-bound acceptor.

We chose Hoechst 33258 as the donor which has a moderate affinity for DNA (K_b =1 × 10^5 M^{-1} and a binding site size of two base pairs) and its fluorescence increases on binding to DNA.[8,16−18] Coumarin 540A (C540A) served as the acceptor which has a high affinity for bovine serum albumin (BSA) (K_b = 1.5 × 10^6 M^{-1}).[12] The donor emission spectrum overlaps with the acceptor absorption spectrum (Figure S1) which is necessary for favorable singlet−singlet energy transfer by the Förster mechanism.[19] The CD spectral titrations indicated that C540A does not bind to DNA and Hoechst 33258 has no affinity for BSA, and the donors and the acceptors can be self-assembled at appropriate sites in the supramolecular complex.

However, BSA is negatively charged at pH 7 and has no affinity for DNA to produce the above supramolecular complex. This problem was solved by chemical modification of the carboxyl groups of BSA via amidation with ethylenediamine (Scheme 1) which resulted in cationized BSA (cBSA) with a net positive charge at pH 7 (Figure S2).[20] The circular dichroism spectrum of cBSA, a measure of protein secondary structure, is superimposable with that of unmodified BSA (Figure S3). Thus, there is little or no structural perturbation due to amidation. Next, we examined cBSA binding to DNA by calorimetry and spectroscopy.

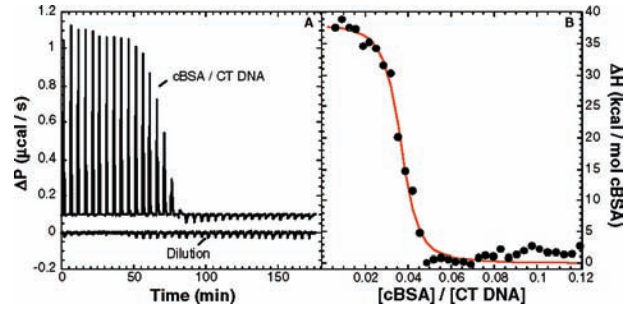


Figure 1. Isothermal titration calorimetric data for the binding of cationized BSA (100 μM) to CT DNA (0−200 μM base pairs) at 298 K in 5 mM TrisHCl, 10 mM NaCl pH 7.2. (A) Raw thermogram with endothermic peaks. (B) Integrated enthalpy change (dots) and the best fit (red line) to the data using a single set, indistinguishable, noninteracting site model.

Titration of cBSA (100 μM) into a solution of calf thymus DNA (CT DNA, 100 μM base pairs, 5 mM TrisHCl, 10 mM NaCl pH 7.2, Figure 1) indicated endothermic, entropy driven binding, despite the fact that binding involved oppositely charged reactants.[21] This is consistent with literature where binding between oppositely charged partners releases solvent/ions from the polar surfaces that are brought in contact and this process is also endothermic.[22]

The binding data were analyzed using a nonlinear least-squares method with different models, and a single set, indistinguishable and noninteracting binding site model[23] gave the best fit. Binding parameters obtained from the fit were K_b = (4.9 ± 4) × 10^7 M^{-1}, stoichiometry of 26 base pairs per cBSA molecule, and ΔH =36 ± 1.7 kcal/mol. The binding site size of 26 base pairs roughly matches with the known hydrodynamic diameter of BSA (21 base pairs).[24] The first example of cBSA binding to DNA to form supramolecular scaffolds is demonstrated, and this success facilitated the construction of DNA-mediated assembly of artificial light harvesting complexes.

Next, we tested the formation of the quaternary complex by titrating Hoechst 33258 (80 μM) bound to CT DNA (200 μM base pairs) with C540A (100 μM) bound to cBSA (100 μM, 5 mM TrisHCl, 10 mM NaCl pH 7.2). The reaction was still

10.1021/ja904551n CCC: $40.75

endothermic ($\Delta H = 12$ kcal/mol), and an estimate of the affinity indicated a decrease in binding constant by an order of magnitude. This decrease in affinity is reasonable, because competitive binding of Hoechst 33258 to DNA diminishes the charge density on DNA and, hence, its affinity for cBSA, but the protein−DNA complex still formed with considerable affinity. Binding of the donor and the acceptor dyes to their respective hosts in the quaternary complex was confirmed by circular dichroism (CD) spectroscopy.

Binding of the donor to the DNA or the acceptor to cBSA resulted in induced-CD spectra, and the CD spectrum of the quaternary complex (40 μM donor, 100 μM DNA base pairs, 60 μM acceptor, and 60 μM cBSA) matched well with the sum of the CD spectra of the DNA-bound donor and protein-bound acceptor (Figure S4). This is the first report of the spontaneous assembly of DNA, protein, donor, and acceptor quaternary systems, and they facilitated the construction of arrays of chromophore assemblies along the DNA helix. Formation of the above quaternary complex was also confirmed by singlet−singlet energy transfer studies, which are described below.

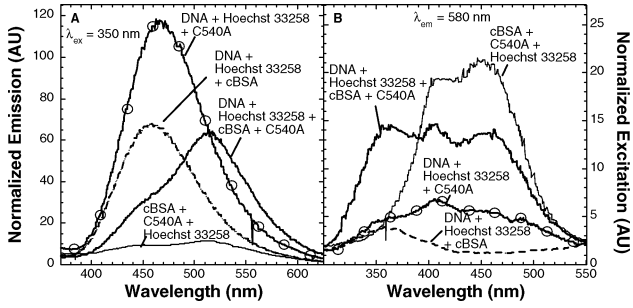


Figure 2. Singlet−singlet energy transfer in the donor−DNA−acceptor−protein quaternary complex (40 μM donor, 100 μM DNA base pairs, 60 μM acceptor, 60 μM cBSA, 20 mM Na_2HPO_4 pH 7.2). (A) Emission spectra of a mixture of Hoechst 33258, CT DNA, cBSA, and C540A (thick line); Hoechst, cBSA, and C540A (thin line); Hoechst, CT DNA, and C540A (thick line with circles); and Hoechst, CT DNA, and cBSA (dashed line). (B) Excitation spectra of Hoechst, CT DNA, cBSA, and C540A (thick line); Hoechst, cBSA, and C540A (thin line); Hoechst, CT DNA, and C540A (thick line with circles); and Hoechst, CT DNA, and cBSA (dashed line) while monitoring at 580 nm. All samples were excited at 350 nm (absorbance <0.15) in a 1 mm cuvette.

The function of the above supramolecular assemblies as light harvesting units was examined in fluorescence studies by exciting the donor at 350 nm such that the acceptor has little or no direct absorption of the excitation light (<10%). Excitation of the DNA-bound donor resulted in strong emission from the protein-bound acceptor (Figure 2A, thick line). Note that micromolar concentrations of the acceptor were adequate to quench >90% of donor emission. In the absence of the acceptor, only donor emission was noted (dashed line) and energy transfer did not occur in the absence of DNA (thin line) or cBSA (thick line with circles). The acceptor emission was increased by 540%, in the presence of the donor, where both the donor and the acceptor are bound to the quaternary complex. Energy transfer is possible at low acceptor concentrations, only if the donor and the acceptor are held in close proximity in the quaternary complex, and such complexes provide new opportunities to construct assemblies of multiple donors and acceptors for light harvesting.

Singlet−singlet energy transfer from donor to the acceptor, in the quaternary complex, was confirmed by recording the excitation spectra (Figure 2B) while monitoring the acceptor emission at 580 nm. The donor has little or no emission at 580 nm, and the excitation spectra should reveal if energy transfer from the donor to the acceptor is successful. The excitation spectra of the quaternary complex (580 nm monitoring) indicated a prominent peak at 358 nm (thick line), which corresponds to the excitation spectrum of Hoechst 33258 (dashed line) recorded in the absence of the acceptor. Therefore, energy transfer from the donor to the acceptor is clearly established. Furthermore, in the absence of DNA (thin line) or cBSA (thick line with circles), the 358 nm peak did not appear in the excitation spectra. This is because the donor does not emit at 580 nm and energy absorbed by the donor is not transferred to the acceptor within the complex. The absence of any one of the four components inhibited energy transfer.

Note that there was no energy transfer between the donor (40 μM) and acceptor (60 μM) in the absence of either DNA or cBSA. The data strongly indicate that the acceptor and the donor are brought within Forster distance in the supramolecular complex. If the quaternary complex is not formed, donor-to-acceptor energy transfer would not be possible. For example, the fluorescence lifetime of the donor bound to the DNA is 1.9 ns,[18] and the acceptor concentration needs to be at least ~1 M (using diffusion limited transfer rate of 5×10^9 M^{-1} s^{-1})[19] to quench 90% of the donor emission. Therefore, the energy transfer process occurs within the quaternary complex.

The central role of cBSA in promoting energy transfer was further tested by replacing cBSA with unmodified BSA, and this inhibited the energy transfer (data not shown), because negatively charged BSA does not bind to negatively charged DNA and, hence, the quaternary complex is not produced. In addition to the cBSA discussed above, we also examined the function of other cationically derivatized BSA molecules. For example, use of ethanolamine and triethylenetetramine in place of enthylenediamine for the chemical modification of BSA (Scheme 1) also resulted in the corresponding cationic BSA samples, and these also formed quaternary complexes with the DNA and facilitated energy transfer (S5).

There was no energy transfer in the absence of either DNA or cBSA, and hence, formation of the protein−DNA complex is essential to promote energy transfer, where both the donor and the acceptor are brought in sufficient proximity within the complex. In support of this explanation, heat denaturation of the quaternary complex at 80 °C for 10 min abolished energy transfer almost completely, which indicates that native-like structures of the biopolymers are essential to support energy transfer. Likewise, 8 M urea, which denatures BSA, completely inhibited the energy transfer. These multiple lines of investigations clearly show that the formation of the quaternary complex is critical for efficient energy transfer from the donor to the acceptor.

Next, we examined the influence of the donor concentration on energy transfer to optimize the transfer efficiency by monitoring the excitation spectra. If the energy transfer occurs between freely diffusing donors and acceptors, the energy transfer efficiency should be independent of donor concentration.[19] On the other hand, if the transfer occurs only in the quaternary complex, then the transfer efficiency would increase with donor concentration and reach a maximum. Further additions of the donor would contribute to free donor, which absorbs light but does not transfer energy to the acceptor, thereby contributing to a net decrease in efficiency. The ratio of the excitation spectral peak intensities at 358 nm (donor absorption peak) to 455 nm (acceptor absorption peak) (I_{358}/I_{455}, 585 nm monitoring) is proportional to the energy transfer efficiency. A

plot of this ratio as a function of donor concentration (100 μM CT DNA, 30 μM cBSA, and 30 μM C540A) showed that the transfer efficiency increases with donor concentration up to 20 μM and then decreases (Figure 3).

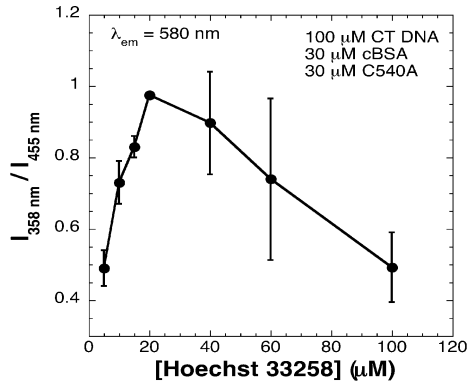


Figure 3. Energy transfer efficiency as a function of donor concentration. Samples contained CT DNA (100 μM base pairs), cBSA (30 μM), and C540A (30 μM) and increasing concentrations of donor (0−100 μM) in 20 mM Na_2HPO_4 pH 7.2 buffer in a 1 mm cuvette.

The initial rapid increase in energy transfer in Figure 3 is due to the accommodation of a increasing number of donor molecules to form the quaternary complex until binding is saturated. Above this concentration, free donors will accumulate which would absorb light but cannot transfer the excitation to the acceptor and, thereby, decrease the overall transfer efficiency. In support of this explanation, when the DNA concentration is increased from 100 to 300 μM base pairs, the peak in the plot of I_{358}/I_{455} vs donor concentration shifted to 40−60 μM donor.

Energy transfer also depended on the acceptor concentration, and increasing C540A concentrations (0−50 μM, at constant DNA, donor, and cBSA concentrations) enhanced quenching. The ratio of donor emission intensity in the absence of acceptor (I_o) to the intensity in the presence of acceptor (I) was plotted as a function of acceptor concentration (Stern−Volmer plot, Figure S6). At 40 μM donor concentration (100 μM DNA base pairs, 60 μM cBSA), the plot was linear up to 20 μM acceptor and had a slope (quenching constant, K_{SV}) of $(4.4 \pm 0.6) \times 10^4$ M^{-1}. If the quenching is diffusion controlled, then the quenching rate constant (k_q, where $k_q = K_{SV}$/lifetime)[19] would be $>10^{13}$ M^{-1} s^{-1}.[18] This value far exceeds diffusion rates of small molecules in solution and strongly supports the idea that energy transfer occurs very rapidly within the protein−DNA quaternary complex, independent of diffusion. The K_{SV} values depended on donor concentration, contrary to what is expected if the energy transfer occurs between freely diffusing donors and acceptors. The K_{sv} values were $(2.9 \pm 0.7) \times 10^4$ and $(1.4 \pm 0.06) \times 10^4$ M^{-1} at 10 and 100 μM donor, respectively. These numerous observations support the interpretation that rapid energy transfer occurs within the supramolecular complex.

For the first time, a proof-of-concept model of artificial light harvesting units based on engineered protein−DNA complexes is demonstrated. All components self-assemble in a predetermined manner to their respective locations within the complex, thereby reducing the synthetic effort needed to construct the unit. This method can be extended to harvest light over a wider range of wavelengths. The versatility of DNA as a supramolecular scaffold will be critical to organize these units along the helix for vectoral energy transfer to artificial reaction centers to achieve efficient capture and conversion of solar energy.

Acknowledgment. We thank the generous financial support from the NSF (DMR-0604815).

Supporting Information Available: Emission and absorption spectra of the donor and the acceptor (S1), the agarose gel of modified BSA (S2), the CD spectrum of cBSA (S3), CD spectra of the assembled components (S4), energy transfer efficiencies of ternary complexes using several modified cationic BSA molecules (S5), and Stern−Volmer plots for the quenching of donor emission (S6). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Balzani, V. Credi, A. Venturi, M. *Molecular Devices and Machines: a Journey into the Nanoworld*; Wiley-VCH: Weinheim, Germany, 2003; pp 132−173.
(2) Nantalaksakul, A.; Reddy, D. R.; Bardeen, C. J.; Thayumanavan, S. *Photosynth. Res.* **2006**, *87*, 133–150.
(3) Balzani, V.; Ceroni, P.; Maestri, M.; Vicinelli, V. *Curr. Opin. Chem. Biol.* **2003**, *7*, 657–665.
(4) Kumar, C. V.; Chaudhari, A.; Rosenthal, G. L. *J. Am. Chem. Soc.* **1994**, *116*, 403–404.
(5) Ward, W. D. *Chem. Soc. Rev.* **1997**, *26*, 365–375.
(6) Li, X.; Sinks, L. E.; Rybtchinski, B.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 10810–10811.
(7) Xu, Q.-H.; Wang, S.; Korystov, D.; Mikhailovsky, A.; Bazan, G. C.; Moses, D.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 530–535.
(8) Murate, S.-I.; Kusba, J.; Piszczek, G.; Gryczynski, I.; Lakowicz, J. R. *Biopolymers* **2000**, *57*, 306–315.
(9) Kakiuchi, T.; Ito, F.; Nagamura, T. *J. Phys. Chem. B* **2008**, *112*, 3931–3937.
(10) Hannestad, J. K.; Sandin, P.; Albinsson, B. *J. Am. Chem. Soc.* **2008**, *130*, 15889–15895.
(11) Benvin, A. L.; Creeger, Y.; Fisher, G. W.; Ballou, B.; Waggoner, A. S.; Armitage, B. A. *J. Am. Chem. Soc.* **2007**, *129*, 5710–5718.
(12) Kumar, C. V.; Duff, M. R. *Photochem. Photobiol. Sci.* **2008**, *7*, 1522–1530.
(13) Nelson, S. M.; Ferguson, L. R.; Denny, W. A. *Mutat. Res.* **2007**, *623*, 24–40.
(14) Kumar, C. V.; Buranaprapuk, A. *J. Am. Chem. Soc.* **1999**, *121*, 4262–4270.
(15) Buranaprapuk, A.; Kumar, C. V.; Jockusch, S.; Turro, N. J. *Tetrahedron* **2000**, *56*, 7019–7025.
(16) Müller, W.; Gautier, F. *Eur. J. Biochem.* **1975**, *54*, 385–394.
(17) Adhikary, A.; Buschmann, V.; Müller, C.; Sauer, M. *Nucleic Acids Res.* **2003**, *31*, 2178–2186.
(18) Cosa, G.; Focsaneanu, K.-S.; McLean, J. R. N.; McNamee, J. P.; Scaiano, J. C. *Photochem. Photobiol.* **2001**, *73*, 585–599.
(19) Turro, N. J. *Modern Molecular Photochemistry*, 2nd ed.; Benjamin/Cummings: Menlo Park, CA, 1978; pp 296−361.
(20) Kumagai, A. K.; Eisenberg, J. B.; Pardridge, W. M. *J. Biol. Chem.* **1987**, *262*, 15214–15219.
(21) Norde, W.; Lyklema, J. *J. Colloid Interface Sci.* **1979**, *71*, 350–366.
(22) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11*, 179–246.
(23) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469–480.
(24) Bohidar, H. B. *Colloid Polym. Sci.* **1989**, *267*, 292–300.

JA904551N