Photoinduced Electron Transfer and Long-lived Charge Separation in Rigid Peptide Architectures

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Two novel architectures, a cyclic dipeptide and a helical nonapeptide rich in α-aminoisobutyric acid (Aib), provide the rigid structural framework for the study of intramolecular, photoinduced electron transfer between two custom-designed redox α-amino acids (donor and acceptor).

A detailed understanding of the principles governing natural photosynthesis[1] has evolved in parallel with the study of artificial molecular systems, designed to mimic key charge-separation functions such as unity charge separation yield, and the retardation of charge recombination.[2] Synthetic electron-transfer model compounds studied to date fall into two categories: totally artificial and biomimetic. In the latter category, the biomimetic character may derive either from the nature of the participating redox units (as in porphyrin–quinone systems[3]) or from the nature of the structural units (proteins,[4] peptides[5]). Here we report our findings on the photoinduced electron transfer observed with a novel donor–acceptor system (Fig. 1) in two different peptide architectures, a cyclic dipeptide and an Aib-rich helical nonapeptide (Fig. 2). These designs feature rigid synthetic redox partners whose biomimetic α-amino acid structure enables them to be readily incorporated into peptides.

The cyclic dipeptide, or dioxopiperazine, structure provides simple access to a rigid, well defined geometry[6,7] ideal for the study of remote interactions between two aromatic side chains.[7,8] The helical peptide approach offers the freedom of incorporating the electronically active components, in the form of α-amino acids, at various distances and orientations by sequence variation and permutation.[9] Our sequence design places the interacting partners close to the middle of the helix, the domain of maximum rigidity and well defined conformation. By exploiting the conformationally constrained amino acid Aib as the main building block, stable helicity (3_{10}) and excellent structural rigidity can be achieved at relatively short peptide lengths (8–10 residues), as has been extensively documented.[10–12]

The required donor and acceptor for our architectures must themselves be α-amino acids. They are designed and synthesized as cyclic α-amino acids (Fig. 1) in order to ensure maximum 3_{10}-helical stability and maintain a well-defined geometrical relationship with no (χ_1, χ_2) torsional degrees of freedom.†

The chromophore was selected to exhibit an ultrafast intersystem crossing to a triplet photoexcited state, whose long lifetime in turn maximizes the distance range accessible in long-range electron transfer. Of a wide range of aromatic carbonyl compounds studied, 2,2,6,7-tetramethyl-1*H*-phenalene-1,3(2*H*)-dione was selected in view of the following photophysical and electrochemical properties: strongly absorbing chromophore, good reversible 1 e$^-$ acceptor,‡ fast $S_1 \rightarrow T_1$ intersystem crossing, unity triplet yield,[14] chemically non-reactive ($\pi\pi^*$) long-lived triplet state at 2.3 eV, good T–T absorption spectrum. The corresponding cyclic amino acid, DkNap, was synthesized in seven steps from naphthalic anhydride.[15]

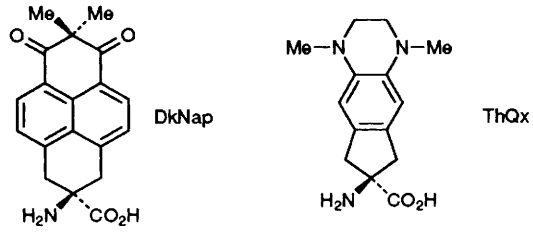

Fig. 1 Molecular structures of acceptor (DkNap) and donor (ThQx) amino acids. The quinoxaline precursor of the ThQx amino acid is called Qx.

A tetrahydroquinoxaline side chain [1,4,6,7-tetramethyl-1,2,3,4-tetrahydroquinoxaline, Fig. 3(*a*)] was selected as the electron-transfer partner of DkNap. This is a powerful donor,[16] giving rise to sufficiently exergonic charge separation. Moreover its photophysical properties are compatible with DkNap.‡ The corresponding cyclic amino acid, ThQx, was prepared in six steps from 4,5-dimethyl-*o*-phenylenediamine. Note that Qx (the quinoxaline analogue) is used instead of ThQx in all intermediate steps of the synthesis; transformation to the active side chain ThQx is achieved after peptide synthesis is completed, through a one-pot reduction-reductive methylation (Scheme 1).

$$\text{}^{t}\text{Boc-DkNap-OH} + \text{H-Qx-OMe} \xrightarrow{\text{i}} \text{}^{t}\text{Boc-DkNap-Qx-OMe} \xrightarrow[\text{iii}]{\text{ii}} \text{c(DkNap-Qx)} \xrightarrow{\text{iv}} \text{c(DkNap-ThQx)}$$

Scheme 1 *Synthesis of the cyclic dimer*: *reagents and conditions*: i, TBTU [2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate], NMM (*N*-methylmorpholine), CH_2Cl_2, 24 h; ii, CF_3CO_2H, CH_2Cl_2, 2 h; iii, heat, cat. glacial AcOH, toluene, 1 h; iv, $NaBH_4$, $(HCHO)_n$ CF_3CO_2H, CH_2Cl_2, 15 min

Dioxopiperazines constructed with mono-α-alkylated amino acids have been studied previously;[6,7,17] here for the

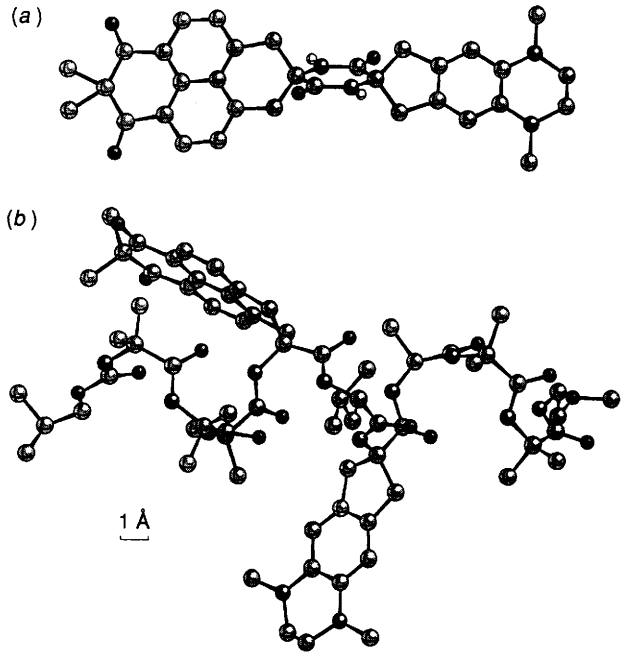

Fig. 2 Minimized structures of (*a*) the cyclic dimer and (*b*) the helical nonamer. In the nonamer (*N*-protected by Boc^i, *C*-protected by $NHCH_2CH_2OMe$), the leucine side chain is seen between the acceptor and the donor. The 3_{10}-helical conformation of the nonamer in its precursor form (DkNap/Qx) has been confirmed by NMR experiments.[11a]

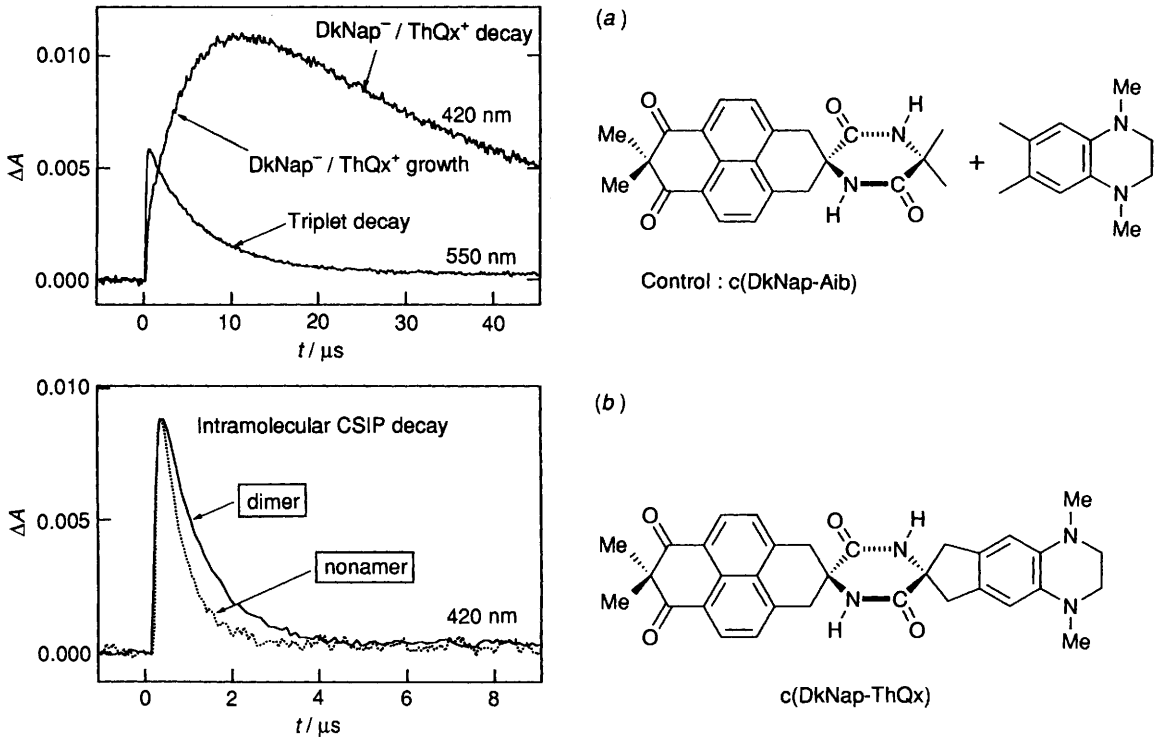

Fig. 3 Transient differential absorption kinetic traces showing (*a*) (bimolecular electron transfer) the quenching of DkNap triplet in the control dimer due to external donor (*ca.* 2×10^{-5} mol dm^{-3}), and the corresponding growth of $DkNap^-/ThQx^+$ followed by non-geminate, diffusion-limited bimolecular charge recombination; (*b*) (intramolecular electron transfer within the cyclic dipeptide) laser-pulse-limited (7 ns) formation of intramolecular CSIP upon photoexcitation of c(DkNap-ThQx) followed by unusually slow intramolecular charge recombination. The dotted trace indicates the CSIP decay in the helical nonamer. Solvent: MeCN.

first time the dioxopiperazine and cyclic α-amino acid strategies are combined to yield a well defined and rigid dispiro molecular geometry, cyclo(DkNap-ThQx).§

In intermolecular electron-transfer experiments the triplet state of the DkNap chromophore in the control dioxopiperazine, cyclo(DkNap-Aib), was efficiently quenched by the external donor [Fig. 3(*a*)]. The pseudo-first-order triplet quenching was accompanied by the appearance of a strong transient absorption at 440 nm characteristic of the radical anion‡ of DkNap, which then decayed, as expected, by second-order kinetics. No processes other than reversible electron transfer were observed.

Cyclo(DkNap-ThQx), upon photoexcitation at 355 nm, exhibits fast electron transfer in polar solvents producing the charge-separated ion pair (CSIP) in unity quantum yield.¶ Charge recombination follows first-order unimolecular kinetics with unusually long lifetimes [1.00 and 3.35 μs in MeCN and tetrahydrofuran (THF), respectively] for a charge-separated state at an edge-to-edge (C_γ–C_γ) distance of *ca.* 8 Å [Fig. 3(*b*)]. Contrasted with the short charge separation times (<7 ns), these long recombination times are well beyond the limits predictable by the Marcus inverted region,[18] and even more so when quantum-inner-sphere modes are taken into account, which serve to increase the rates of large exoergicity charge recombination.[19] We believe that this very slow recombination is a consequence of the persistence of the triplet-correlated radical-ion-pair character in the charge-separated state which results in the corresponding forbiddenness of the charge recombination back to the ground-state singlet. In the marginally polar solvent toluene the charge separation kinetics were cleanly time-resolved at −80 °C, where a 25 ns rise-time was observed, leading to an equilibrium between the CSIP and the DkNap triplet.‖ The charge-separation rate constant, k_{et}, is then a component of this 25 ns relaxation time [25 ns = $(k_{et} + k_{-et})^{-1}$] and is calculated to be 0.9×10^7 s^{-1}.

Following the cyclic photoactive dimer, the helical nonapeptide ^{t}Boc(Aib)$_3$-DkNap-Leu-ThQx-Ala(Aib)$_2$NHR§ was synthesized, containing the two electronically active amino acids as next nearest neighbours with a leucine as the intervening residue. Extensive 1-D (solvent perturbation)[11*a*] NMR studies on the precursor ^{t}Boc(Aib)$_3$-DkNap-Leu-Qx-Ala(Aib)$_2$NHR, and 2-D (ROESY) NMR studies[11*b*] on the corresponding control nonamer, ^{t}Boc(Aib)$_3$-DkNap-LeuAib-Ala(Aib)$_2$NHR, unambiguously prove the 3_{10}-helical structure of the peptide. Immediately after photoexcitation of the DkNap chromophone the spectrum of the charge separated product state is observed, indicating fast forward electron transfer (<7 ns) and unity charge separation quantum yield. The lifetime of the resultant CSIP in the nonamer is 0.57 μs in MeCN [Fig. 3(*b*)]. The edge-to edge donor–acceptor distances are very similar in both molecules (*ca.* 8 Å); however, the difference in the intervening framework clearly influences the persistence of the CSIP. These preliminary data on the nonamer demonstrate the wide time window for intrahelical electron transfer offered by DkNap and ThQx. A charge separation rate 1000 times slower would still lead to a high charge-separation quantum yield. This pair of rigid amino acids is ideally suited for probing longer range peptide-mediated electron-transfer interactions in families of peptides as a function of distance, orientation and intervening medium.

Our experiments have demonstrated two different architectures suitable for the study of electron transfer between electronically active amino acids. Dioxopiperazine formation when combined with rigid side-chain amino acids provides a simple and powerful methodology yielding an extremely well defined geometry. The Aib-rich 3_{10}-helical peptide enables the preparation of a molecular optical rail, ideal for the exploration of electronic interactions between diverse amino acids at variable intercomponent distances and orientations. The unusually large ratio of charge separation rate to charge

recombination rate is a rare and desirable property of molecular systems designed for photo-induced charge separation. We define this ratio as the 'radical ion pair accumulation ratio',[20] and are pursuing additional studies to determine its variation and further optimization.

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Footnotes

† It has been shown that 1-aminocyclopentane-1-carboxylic acid and 1-aminocyclohexane-1-carboxylic acid homopeptides form very stable 3_{10}-helices.[13]

‡ Redox potentials *vs.* SCE in MeCN: $E_{1/2}$(DkNap) = −1.51 V, $E_{1/2}$(ThQx) = +0.14 V. The S_1 (3.7 eV) and T_1 (2.8 eV) states of ThQx are higher in energy than the corresponding states of DkNap making excitation transfer unfavourable. The DkNap radical anion has been generated independently in a spectroelectrochemical cell and its absorption spectrum matches the one observed in the electron-transfer experiment. The absorption of the ThQx radical cation is overall much weaker; it has a maximum at 423 nm.

§ The photoactive dimer and nonamer as well as their DkNap/Qx precursors gave satisfactory NMR spectra; both precursors were also verified by mass spectra (high resolution in the dimer case).

¶ The efficient intersystem crossing of DkNap along with the fast charge-separation rate of the dimer indicate a unity charge separation quantum yield. This was further verified by picosecond transient absorption experiments[20] in which the DkNap triplet decay and the simultaneous growth of the CSIP were observed in acetonitrile. The intersystem crossing rate is much faster than any other singlet decay pathway and similarly the charge separation is much faster than any other triplet decay pathway.

‖ The equilibrium constant, [CSIP]/[c(^{3}DKNap-ThQx)], in toluene is *ca.* 0.3, whereas in highly polar solvents formation of CSIP is quantitative. The nanosecond transient absorption spectrum of the dimer in toluene shows distinct absorption components at 440 and 560 nm corresponding to the CSIP and the DkNap triplet state, respectively. The two spectral components decay with identical kinetics indicating fast equilibrium and allowing the extraction of the equilibrium constant. Molar absorption coefficients: ε(DkNap triplet at 560 nm) = 5.2×10^3 dm^3 mol^{-1} cm^{-1} and ε(CSIP at 440 nm) = 18.2×10^3 dm^3 mol^{-1} cm^{-1}.

References

1 J. Jortner and B. Pullman, ed., *Perspectives in Photosynthesis*, Kluwer, Dordrecht, 1990.

2 V. Balzani and F. Scandola, *Supramolecular Photochemistry*, Ellis Horwood, New York, 1991; D. Gust and T. A. Moore, ed., *Covalently Linked Donor-Acceptor Species for Mimicry of Photosynthetic Electron And Energy Transfer*, (*Tetrahedron Symposia-in-Print*) 1989, **45**, 4669–4912; J. S. Connolly and J. R. Bolton in *Photoinduced Electron Transfer*, ed. M. A. Fox and M. Chanon, Elsevier, Amsterdam, 1988, Part D, pp. 303–393; D. Gust, T. A. Moore and A. L. Moore, *Acc. Chem. Res.*, 1993, **26**, 198.

3 A. D. Joran, B. A. Leland, P. M. Felker, A. H. Zewail, J. J. Hopfield and P. B. Dervan, *Nature*, 1987, **327**, 508; M. Antolovich, P. J. Keyte, A. M. Oliver, M. N. Paddon-Row, J. Kroon, J. W. Verhoeven, S. A. Jonker and J. M. Warman, *J. Phys. Chem.*, 1991, **95**, 1933; J.-Y. Liu and J. R. Bolton, *J. Phys. Chem.*, 1992, **96**, 1718; S. Knapp, T. G. Murali Dhar, J. Albaneze, S. Gentemann, J. A. Potenza, D. Holten and H. J. Schugar, *J. Am. Chem. Soc.*, 1991, **113**, 4010; J. K. Delaney, D. C. Mauzerall and J. S. Lindsey, *J. Am. Chem. Soc.*, 1990, **112**, 957; C. A. Hunter, J. K. M. Sanders, G. S. Beddard and S. Evans, *J. Chem. Soc., Chem. Commun.*, 1989, 1765.

4 D. S. Wuttke, M. J. Bjerrum, J. R. Winkler and H. B. Gray, *Science*, 1992, **256**, 1007; A. Willie, P. S. Stayton, S. G. Sligar, B. Durham and F. Millett, *Biochemistry*, 1992, **31**, 7237.

5 S. S. Isied, in *Metal Ions in Biological Systems*, ed. H. Sigel and A. Sigel, Marcel Dekker, New York, 1991, vol. 27, pp. 1–56; M. Sisido, R. Tanaka, Y. Inai and Y. Imanishi, *J. Am. Chem. Soc.*, 1989, **111**, 6790; M. Faraggi, M. R. DeFellipis and M. H. Klapper, *J. Am. Chem. Soc.*, 1989, **111**, 5141; K. S. Schanze and K. Sauer, *J. Am. Chem. Soc.*, 1988, **110**, 1180.

6 T. Yamazaki, K. Nunami and M. Goodman, *Biopolymers*, 1991, **31**, 1513 and references therein.

7 G. Basu, M. Kubasik, D. Anglos and A. Kuki, *J. Phys. Chem.*, 1993, **97**, 3956.

8 G. Basu, M. Kubasik, D. Anglos, B. Secor and A. Kuki, *J. Am. Chem. Soc.*, 1990, **112**, 9410.

9 G. Basu, D. Anglos and A. Kuki, *Biochemistry*, 1993, **32**, 3067.

10 G. Basu, K. Bagchi and A. Kuki, *Biopolymers*, 1991, **31**, 1763; G. Basu and A. Kuki, *Biopolymers*, 1993, **33**, 995.

11 V. Bindra and A. Kuki, (*a*) *Int. J. Pept. Prot. Res.*, submitted for publication; (*b*) manuscript in preparation.

12 I. L. Karle and P. Balaram, *Biochemistry*, 1990, **29**, 6747; C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. DiBlasio, P. Grimaldi, F. Lelj, V. Pavone and C. Pedone, *Macromolecules*, 1985, **18**, 895; G. R. Marshall, E. E. Hodgkin, D. A. Langs, G. D. Smith, J. Zabrocki and M. Leplawy, *Proc. Natl. Acad. Sci. USA*, 1990, **87**, 487.

13 M. Crisma, G. M. Bonora, C. Toniolo, E. Benedetti, A. Bavoso, B. DiBlasio, V. Pavone and C. Pedone, *Int. J. Biol. Macromol.*, 1988, **10**, 300; M. Crisma, G. M. Bonora, C. Toniolo, A. Bavoso, E. Benedetti, B. DiBlasio, V. Pavone and C. Pedone, *Macromolecules*, 1988, **21**, 2071.

14 D. W. Boldridge, B. L. Justus and G. W. Scott, *J. Chem. Phys.*, 1984, **80**, 3179, and references therein. The DkNap chromophore exhibits extremely low fluorescence emission (quantum yield ≤0.002) as well as fast intersystem crossing (<10 ps).[20]

15 S. R. Kotha, D. Anglos and A. Kuki, *Tetrahedron Lett.*, 1992, **33**, 1569.

16 S. F. Nelsen, E. L. Clennan, L. Echegoyan and L. A. Grezzo, *J. Org. Chem.*, 1978, **43**, 2621.

17 D. B. Bivin, S. Kubota, R. Pearlstein and M. F. Morales, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 6791; S. Egusa, J. Takagi, M. Sisido and Y. Imanishi, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 2195.

18 R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta*, 1985, **811**, 265. Typical solvent reorganization energy (λ) for acetonitrile: 1.25 eV.

19 N. R. Kestner, J. Logan and J. Jortner, *J. Phys. Chem.*, 1974, **78**, 2148; J. Ulstrup and J. Jortner *J. Chem. Phys.*, 1975, **63**, 4358; S. Efrima and M. Bixon, *Chem. Phys.*, 1976, **13**, 447; R. P. van Duyne and S. F. Fischer, *Chem. Phys.*, 1974, **5**, 183.

20 D. Anglos, B. Li, K. S. Peters and A. Kuki, manuscript in preparation.