Thermal Control over the Extent of Photoinduced Electron Transfer in Helical Oligopeptides

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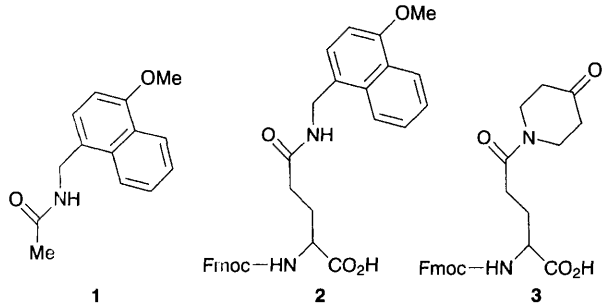
Time-resolved fluorescence studies of an electron donor–acceptor modified helical oligopeptide, in acetonitrile, show a biexponential decay with a short lifetime component attributed to rapid photoinduced electron transfer (PET), which increases from 15% of the total fluorescence at −40 °C to 58% on warming to 0 °C and then more gradually to 67% at 60 °C.

Molecular systems that respond to specific environmental changes occurring at a molecular level and signal the response at a macroscopic level are of great interest because of their potential applications in chemical sensing and molecular electronics. Of particular interest are systems of a generic nature where simple changes to the molecular structure can be used to alter the specificity of response without changing the transduction system. We have been interested in using the well defined secondary structures of oligopeptides as a route to conformational molecular switches where different states can be probed optically by monitoring the extent of intramolecular electron transfer quenching of an internal fluorescent probe. The ability to introduce a wide range of functionality into the peptide chain provides a potential mechanism for directing switching between conformational states using external stimuli such as temperature, light, pH, dielectric, metal ions and molecular binding. Here we describe our initial studies on an organic soluble helical oligopeptide that exhibits thermal gating of the efficiency of electron transfer.

The modified glutamic acid residues, (Fmoc–Don–OH) **2** and (Fmoc–Acc–OH) **3**, Fig. 1, were synthesised by coupling the appropriate amine derivative to the side chain carboxyl of Fmoc–Glu(OH)–OBu^t using HBTU activation followed by deprotection using TFA.† The twelve residue oligopeptides **4** and **5** were made *via* conventional solid-state peptide synthesis on a modified Rink type resin using Fmoc chemistry and PyBOP coupling protocols.‡ The oligopeptides were purified to homogeneity using reversed-phase HPLC and characterised *via* electrospray mass spectrometry.§ The peptides were identical in sequence except for the 9th residue. Oligopeptide **4** had alanine at this position while oligopeptide **5** contained the modified glutamic acid residue (Acc) in which the ketone of the pendant piperidone group acts as a weak electron acceptor. In both peptides the 5th residue was the modified glutamic acid (Don) with a pendant methoxynaphthalene group that serves as a good fluorescent donor for studies of photoinduced electron transfer (PET).¶ The lowest absorption band of the ketone (I_{max} = 288 nm) and the emission band of methoxynaphthalene (I_{max} = 358 nm) exhibit negligible spectral overlap. Hence, with this donor–acceptor combination quenching of fluorescence by energy transfer is minimal and electron transfer is expected to be the dominant process.[1] A further design feature of the oligopeptides was the inclusion of α-aminoisobutyric acid (Aib) at the 3rd, 7th and 11th positions in the sequence in order to promote helix formation.[2,3]



1 2 3

Acetyl–Ala–Aib–Ala–Ala–Leu–Aib–Ala–Don–Ala–Aib–Leu–Ala–NH_2 **4**

Acetyl–Ala–Aib–Ala–Acc–Leu–Aib–Ala–Don–Ala–Aib–Leu–Ala–NH_2 **5**

Fig. 1 Structure of model donor **1**, modified amino acids **2**, **3** and peptide sequences **4**, **5**

The solution state conformation of the oligopeptides was investigated in acetonitrile using circular dichroism and the spectra obtained for peptide **5** at 0, 20 and 45 °C are shown in Fig. 2. The CD spectrum is characteristic of a helical structure[4] and only a small variation in intensity over the temperature range was observed. The CD spectra were analysed using the CONTIN procedure of Provencher and Glöckner[5] and at 20 °C indicated that the peptide was folded into 37% α-helix, 52% β-sheet and 11% 'other' with no random coil present. The presence of a significant level of β-sheet appears unlikely in view of the low concentrations used (10^{-4} mol dm^{-3}), the high solubility in organic solvents and the pronounced conformational preferences of Aib residues. However, the analysis program was designed primarily for protein studies and does not include a 3_{10} helix structural motif in the analysis. 3_{10} Helices are reported to give a similar but less intense CD spectrum to an α-helix[6] thus it is possible the peptides are a mixture of 3_{10} and α-helices. Recent NMR studies of Aib containing peptides have shown that for certain sequences interconversion between the two helical forms can occur at room temperature.[7]

A time-resolved fluorescence study of the peptides was carried out in degassed acetonitrile at 20 °C. The fluorescence decay of peptide **4** was best described using a single exponential fluorescence function giving a lifetime of 7.57 ± 0.03 ns. This compares closely to the 7.61 ± 0.03 ns fluorescence lifetime of the model compound **1** and shows that the oligopeptide backbone does not affect the fluorescence behaviour. For peptide **5** the fluorescence decay behaviour was best modelled using a biexponential function.†† The minor component present at 36% of the total fluorescence had a lifetime of 7.06 ± 0.13 ns, only slightly shorter than the lifetimes of **1** and **4**. The remaining 64% of the fluorescence was found to be strongly quenched with a lifetime of 3.23 ± 0.09 ns. This shorter component can be

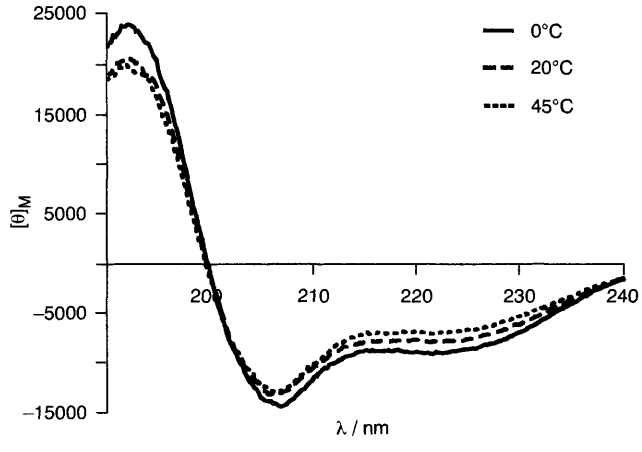


Fig. 2 CD spectrum for oligopeptide **5** in acetonitrile

assigned to oligopeptide where photoinduced electron transfer between the methoxynaphthalene donor and the ketone acceptor occurred.

To obtain further information a temperature dependent study of the fluorescence behaviour was carried out. It was found that at all temperatures the decay curves were best modelled by a biexponential function. Fig. 3 shows the interesting variation in intensity of the short-lived component on heating from −40 to 60 °C. Measurements were also taken on cooling to check for hysteresis. At the lowest temperature less than 20% of the fluorescence was strongly quenched; however, on warming the amount of the short-lived component increased rapidly, reaching 58% at 0 °C. In contrast a much smaller change occurred over the range from 0 to 60 °C. This temperature study provides strong evidence that a single compound is involved in the fluorescence process and impurities are not a factor. The lack of hysteresis shows the populations associated with each fluorescing component are readily interconvertible and that irreversible thermal inactivation of the oligopeptide does not occur.

The lifetime of the short-lived component relative to the unquenched fluorescence (obtained from peptide **4**) was used to calculate the electron transfer rate at each temperature and the data used to construct the Arrhenius plot shown in Fig. 4. The activation energy for electron transfer, obtained from the gradient of the best fit line, was 5.7 ± 0.9 kJ mol^{-1}. This modest value is of the order of a rotational barrier and suggests that the oligopeptide accesses a near optimum conformation for electron transfer.

Combining these results leads to the interesting conclusion that initially, as the temperature is raised from −40 °C, the fraction of molecules exhibiting quenching increases rapidly while over the same range the rate of electron transfer is almost invariant. We believe that this unusual behaviour is a consequence of the size and well defined secondary structure of these oligopeptides. Initial molecular dynamic calculations show that in such systems there are only a limited number of dynamic pathways describing the transition between conformations. Thus, even where there is no significant energy barrier, between for example a conformation where electron transfer occurs and one where it is not favoured, quenching may not be observed because the time required for traversing the pathway between the states is longer than the fluorescence lifetime. Using this reasoning the increase in population of the quenched species with temperature can be explained in terms of an increase in mobility of the peptide and a reduction in the transition time between different conformational states.

The nature of the optimum conformation for electron transfer is as yet unknown, but the results suggest that it lies within a steep-sided energy well. The relatively small changes observed in the CD spectrum with temperature indicate that the conformation is at least partially helical. However, the possibility that electron transfer takes place from a short-lived conformation not observed in the CD measurements cannot be ruled out. We are currently using NMR to probe further the conformational behaviour of these oligopeptides.

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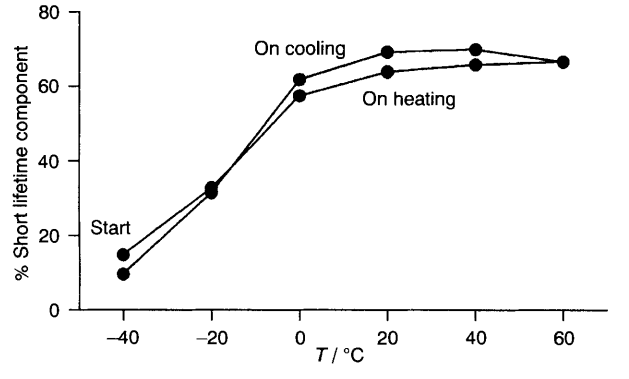

Fig. 3 Change in the percentage of the short fluorescence lifetime component of **5** with temperature

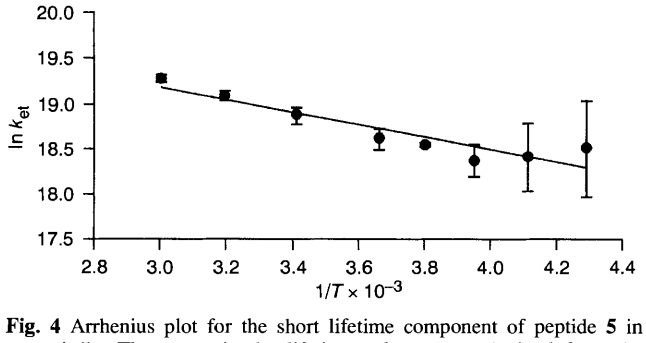

Fig. 4 Arrhenius plot for the short lifetime component of peptide **5** in acetonitrile. The errors in the lifetime values were obtained from the reconvolution analysis and are equivalent to three standard deviations.

Footnotes

† The oligopeptides were synthesised using a Novasyn Crystal solid phase peptide synthesiser, Novasyn PR500 resin, Fmoc protected amino acids and PyBOP coupling chemistry. Each residue was double coupled using a 2.5-fold excess of amino acid. Cleavage was with 10% TFA in CH_2Cl_2 for 90 mins and purification was *via* reversed-phase HPLC on a C_8 column using a MeOH/H_2O gradient.

‡ All compounds and intermediates gave satisfactory NMR data and elemental analysis.

§ ES–MS data. Peptide **4**, *m/z* = 1273.5, found at *m/z* = 1312.3 (m + K^+), 1296.4 (m + Na^+), 1274.2 (m + H^+), 659.8 (m + 2 Na^+), 656.2 (m + H^+ K^+), 649.0 (m + H^+ Na^+), 637.7 (m + 2H^+).

Peptide **5**, *m/z* = 1404.6, found at *m/z* = 1443.2 (m + K^+), 1427.0 (m + Na^+), 1405.0 (m + H^+), 725.5 (m + 2 Na^+), 722.5 (m + H^+ K^+), 714.5 (m + H^+ Na^+), 703.4 (m + 2H^+).

¶ Using $\Delta G° = [E_{ox}(\mathbf{2}) - E_{red}(\mathbf{3})] - E_{0,0}(\mathbf{2})$, where $E_{ox}(\mathbf{2})$ and $E_{red}(\mathbf{3})$ were obtained from the peak potential of the irreversible oxidation and reduction waves in acetonitrile and $E_{0,0}(\mathbf{2})$ was obtained from analysis of the absorption and emission spectra. $\Delta G°$ = ([1.28] − [−2.02]) − 3.80 = −0.50 eV = −48.3 kJ mol^{-1}.

†† Good fits were judged by the chi-squared value (< 1.2) and weighted residuals. For peptide **5** at 20 °C the single exponential chi-squared value was 6.07 compared with 1.03 obtained using a biexponential (sum of two exponentials) fit.

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