# INTRAMOLECULAR ELECTRONIC EXCITATION ENERGY TRANSFER IN DERMORPHINE AND ITS ANALOGUES

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## Summary

The fluorescence quantum yields of dermorphine and its analogues Ala[1]-dermorphine and Ala[5]-dermorphine were measured at two different excitation wavelengths $\lambda_1 = 280$ nm and $\lambda_2 = 260$ nm. Hence the efficiencies of the excitation energy transfer from phenylalanine to tyrosine in the above oligopeptides were determined. It has been found from the results obtained that the mean phenylalanine–tyrosine separation in Ala[5]-dermorphine is 10% longer than that in Ala[1]-dermorphine. Moreover, good agreement was found between the quantum efficiency of energy transfer for dermorphine and the value calculated from kinetic equations using the transfer efficiencies determined for Ala[1]- and Ala[5]-dermorphine analogues.

## 1. Introduction

Investigations of the efficiency of excitation energy transfer in polypeptides have undergone many developments in recent years owing to the possibility, in principle, of determining the intramolecular donor–acceptor distances [1 - 3]. Numerous investigations of this problem in Leu- and Met-enkephalin and their analogues have been reported [4 - 8]. A knowledge of intramolecular donor–acceptor separations in polypeptides is in many cases helpful in determining the conformation of these macromolecules [9].

We have recently described the synthesis of dermorphine, which is a peptide exhibiting strong opiate effects [10]. The sequence of this peptide, which was isolated for the first time from the skin of the South American frog *Phyllamedusa*, is H—Tyr—D—Ala—Phe—Gly—Tyr—Pro—Ser—$NH_2$ [11].

From the viewpoint of the excitation energy transfer, the dermorphine molecule is a system with one donor (phenylalanine (Phe) in position 3) and two alternative acceptors (tyrosine (Tyr) in positions 1 and 5). The measurement of energy transfer in dermorphine does not itself enable the Tyr[1]–Phe and Phe–Tyr[5] distances $R_1$ and $R_2$ to be determined. In order to

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find $R_1$ and $R_2$, it is necessary to investigate the intramolecular excitation energy transfer in the dermorphine analogues Ala$^5$-dermorphine (H—Tyr—D—Ala—Phe—Gly—Ala—Pro—Ser—$NH_2$) and Ala$^1$-dermorphine (H—Ala—D—Ala—Phe—Gly—Tyr—Pro—Ser—$NH_2$) in which one donor and one acceptor are involved.

## 2. Materials and methods

Dermorphine [10] and its analogues Ala$^1$- and Ala$^5$-dermorphine [12] were obtained by solid phase synthesis using the Merrifield method [13] and were purified by gel filtration on G-10 Sephadex. The tripeptide H—D—Ala—Phe—Gly—OH (melting point, 408–410 K; $[\alpha]_D^{20} = 7.4°$) was obtained in solution using the standard method. The concentrations of the aqueous solutions (pH 6.8) of the substances investigated were $2 \times 10^{-4}$ M in all cases. Absorption spectra were obtained using a Beckman model 25 spectrophotometer. The fluorescence spectra were recorded using the spectrofluorometer described by Kawski *et al.* [14]. The energy transfer efficiencies $T$ were found from the sensitized fluorescence of the acceptor Tyr.

## 3. Results and discussion

According to the theory of non-radiative transfer of excitation energy [15, 16], the donor–acceptor separation depends on the transfer efficiency $T$:

$$R = R_0 C^{1/6} \tag{1}$$

where $R_0$ is the critical distance and $C = 1/T - 1$. $R_0$ is given by the following expression:

$$R_0^6 = \frac{9\kappa^2(\ln 10)\eta_D}{128\pi^5 n^4 N'} \int_0^\infty f_D(\tilde{\nu})\epsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4} \tag{2}$$

where $\tilde{\nu}$ is the wavenumber, $\epsilon_A(\tilde{\nu})$ is the decadic molar extinction coefficient of the acceptor, $f_D(\tilde{\nu})$ is the relative fluorescence intensity of the donor, $N'$ is the number of molecules per millimole, $n$ is the refractive index of the intervening medium, $\kappa^2$ is the orientation factor, $\eta_D$ is the fluorescence quantum yield of the donor and the integral represents the overlap of the absorption spectrum of the acceptor and the fluorescence spectrum of the donor.

The tripeptide H—D—Ala—Phe—Gly—OH, in which the Phe environment is identical with that in dermorphine and its analogues, acted as the energy donor. The donor quantum yield $\eta_D$ in water was determined relative to that of Tyr which was used as the standard quantum yield ($\eta_s = 0.14$) [17]. The value of $\eta_D$ obtained in this way was 0.037 which is close to the

quantum yield of Phe bonded in Leu-enkephalin ($\eta_D = 0.035$) [8]. The values obtained for the overlap integral ($3.6 \times 10^{-16}$ cm$^6$ mol$^{-1}$) and the critical distance (11.6 Å) are in good agreement with those reported for other oligopeptides in which Phe is the donor and Tyr is the acceptor of the excitation energy.

In order to determine the critical distance $R_0$ (eqn. (2)) the relative orientations of the transition dipole moments in the donor and acceptor, which are described by the orientation factor $\kappa^2$ which varies from zero to 4, should be known. A comprehensive analysis of the effect of the mutual orientation of the electronic transition moments of the donor and the acceptor on the transfer efficiency of the excitation energy has been given by Dale and coworkers [18, 19]. In a situation of dynamic random orientation $\kappa^2$ has a value of 2/3. A significant problem in determining the exact value of $\kappa^2$ for oligopeptide solutions is their dynamic structure which is manifested by internal motion on the subnanosecond scale [20]. In solutions of low viscosity, considerable mobility of the aromatic rings of Phe and Tyr should be expected in addition to the flexibility of the main skeleton. Therefore the separation $R$ determined from eqn. (1) should be treated as a mean value. There is some evidence that the true value of $\kappa^2$ for the systems investigated in aqueous solutions differs only slightly from 2/3. However, even if the orientation is fixed the uncertainty in the orientation factor is substantially reduced for the Phe–Tyr donor–acceptor pair because the polarization of Tyr is mixed in the wavelength region of spectral overlap. In view of the considerations in ref. 21 the low value of the degree of polarization of Tyr allows a value of $\kappa^2 = 2/3$ to be assumed in the calculations of $R_0$. $^{13}$C spin–lattice relaxation investigations may also indirectly justify the use of $\kappa^2 = 2/3$. They imply that in the case of oligopeptides the aromatic ring in Phe has considerable mobility with respect to the main peptide backbone [22]. However, we believe that for the dermorphine analogues investigated it is only necessary to determine the relative magnitudes of $R_1$ and $R_2$; the absolute values of these distances are of minor importance. Therefore in further considerations it is important to assume the same value of $\kappa^2$ for dermorphine and its analogues. This is supported by the identical environments of the donor and acceptor in dermorphine and its analogues.

The transfer efficiencies $T$ can be determined by measuring the fluorescence quantum yields of the oligopeptides and the free acceptor Tyr at various excitation wavelengths. The surface areas under the fluorescence spectral distribution curves for the free acceptor and the oligopeptide can be written as

$$P_0 = \alpha_A^\circ \eta_A^\circ \tag{3}$$

and

$$P = \alpha_D(1 - T)\eta_D + \alpha_A \eta_A + \alpha_D T \eta_A \tag{4}$$

respectively where $\eta_D$ is the fluorescence quantum yield of the donor bound in the oligopeptide, $\eta_A^\circ$ and $\eta_A$ are the fluorescence quantum yields for the

free acceptor and the acceptor bound in the oligopeptide respectively, $\alpha_A^\circ$ is the fraction of light absorbed on excitation by the free acceptor, and $\alpha_D$ and $\alpha_A$ are the fractions of the excitation light absorbed by the donor and acceptor in an oligopeptide respectively.

When the acceptor fluorescence only is observed (the fluorescence spectra of Phe and Tyr are sufficiently widely separated and the fluorescence quantum yield of Tyr is many times higher than that of Phe) we obtain the following relations: at an excitation wavelength $\lambda_1 = 280$ nm which is outside the donor absorption region (Fig. 1)

$$\frac{P(\lambda_1)}{P_0(\lambda_1)} = \frac{\alpha_A(\lambda_1)}{\alpha_A^\circ(\lambda_1)} \frac{\eta_A}{\eta_A^\circ} \tag{5}$$

and at an excitation wavelength $\lambda_2 = 260$ nm which is inside the donor absorption region

$$\frac{P(\lambda_2)}{P_0(\lambda_2)} = \frac{\alpha_A(\lambda_2)\eta_A + \alpha_D(\lambda_2)\eta_A T}{\alpha_A^\circ(\lambda_2)\eta_A^\circ} \tag{6}$$

From eqns. (5) and (6) we obtain for the transfer efficiency

$$T = \frac{P_0(\lambda_1)}{P(\lambda_1)} \frac{P(\lambda_2)}{P_0(\lambda_2)} \frac{\alpha_A(\lambda_1)}{\alpha_A^\circ(\lambda_1)} \frac{\alpha_A^\circ(\lambda_2)}{\alpha_D(\lambda_2)} - \frac{\alpha_A(\lambda_2)}{\alpha_D(\lambda_2)} \tag{7}$$

In order to determine $T$, it is sufficient to measure the fluorescence of the system studied and that of the free acceptor at two different excitation wavelengths under the same experimental conditions. The fluorescence quantum yield of the acceptor Tyr was the same at the two excitation wavelengths. The values of $\alpha_A$, $\alpha_A^\circ$ and $\alpha_D$ can be found from the absorption laws and by using the absorption data for the solutions examined. For dilute solutions (the case dealt with in the present paper) the values of $\alpha_A$, $\alpha_A^\circ$ and $\alpha_D$ can be replaced by the respective absorbances. The absorption spectra of the oligopeptides studied at wavelengths exceeding 240 nm are the superposition of the Phe and Tyr absorption spectra, which is

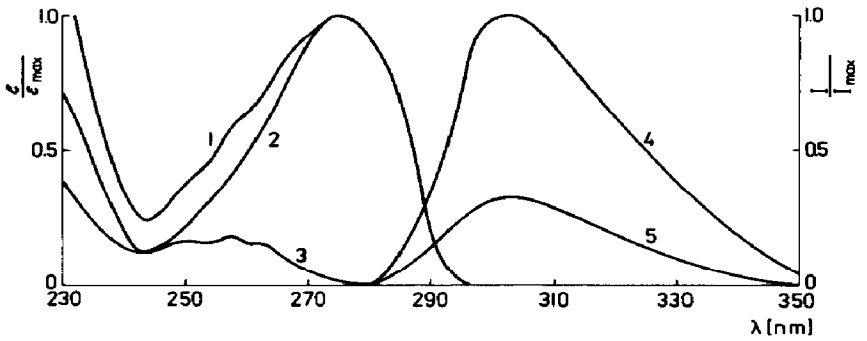


Fig. 1. Absorption spectra of Ala$^1$-dermorphine (curve 1), Tyr (curve 2) and Phe (curve 3); fluorescence spectra of Tyr (curve 4) and Ala$^1$-dermorphine (curve 5). The absorption spectra of Phe and Tyr are related to the absorption of Ala$^1$-dermorphine and the emission spectrum of Ala$^1$-dermorphine is related to the fluorescence spectrum of Tyr.

illustrated in Fig. 1 for Ala[1]-dermorphine (a similar situation occurs for the remaining oligopeptides investigated).

The fluorescence spectral distribution of dermorphine and its analogues is identical with that of Tyr, and hence we can assume that the chromophores are also independent in the excited state. The fluorescence quantum yield of dermorphine and its analogues (Table 1) is more than a factor of 3 lower than that of free Tyr. This is due to the effect of the peptide bonds on the chromophores, which causes a reduction by a factor of 3 - 4 in the fluorescence quantum yield [23]. Table 1 also gives the values determined for the transfer efficiencies $T$ as well as the separations $R_1$ and $R_2$. The results obtained imply that the separation between Tyr[1] and Phe slightly exceeds that between Phe and Tyr[5].

The transfer efficiency $T$ in dermorphine is substantially higher than that in its analogues. This seems reasonable, since the same donor has two acceptor residues in its vicinity in dermorphine, whereas there is only one acceptor residue in Ala[1]- and Ala[5]-dermorphine.

The transfer efficiencies for Ala[5]- and Ala[1]-dermorphine are $T_1$ and $T_2$ respectively:

$$T_1 = \frac{k_{T_1}}{k_f + k_n + k_{T_1}} \tag{8}$$

$$T_2 = \frac{k_{T_2}}{k_f + k_n + k_{T_2}} \tag{9}$$

For dermorphine itself, the transfer efficiency is given by

$$T = \frac{k_T}{k_f + k_n + k_T} \tag{10}$$

where $k_f$ is the rate constant of radiative transition in the donor, $k_n$ is the rate constant of non-radiative transition and $k_T$ is the rate constant of the energy transfer from the donor to the acceptor.

If the processes of excitation energy transfer from Phe to Tyr[1] or Tyr[5] in dermorphine are independent, $k_T = k_{T_1} + k_{T_2}$ and $T$ is given by

TABLE 1

Relative fluorescence quantum yields, the efficiencies of the excitation energy transfer and the distances between Phe and Tyr in dermorphine and its analogues

| *Substance* | $\eta_A^\circ/\eta_A$ | $T$ (±0.02) | $R^a$ (Å) |
|---|---|---|---|
| Dermorphine | 3.26 | 0.90 | — |
| Ala[1]-dermorphine | 3.14 | 0.82 | 9.0 |
| Ala[5]-dermorphine | 3.28 | 0.76 | 9.6 |

[a] $\kappa^2 = 2/3$.

$$T = \frac{k_{T_1} + k_{T_2}}{k_f + k_n + k_{T_1} + k_{T_2}} \quad (11)$$

The following relation can be obtained from expressions (8), (9) and (11):

$$C = \frac{C_1 C_2}{C_1 + C_2} \quad (12)$$

where $C = 1/T - 1$, $C_1 = 1/T_1 - 1$ and $C_2 = 1/T_2 - 1$, and hence

$$T = \frac{1}{1 + C} \quad (13)$$

Substituting the respective values of $T_1$ and $T_2$ for Ala$^5$- and Ala$^1$-dermorphine, we obtain $T = 0.89$ from eqns. (12) and (13). The agreement of this value with that determined directly for dermorphine (see Table 1) confirms the validity of the assumption that the individual energy transfer processes in dermorphine are independent. Good agreement between the calculated and directly measured efficiency $T$ of the energy transfer for dermorphine indicates indirectly that the conformations of the Ala$^1$- and Ala$^5$-dermorphine analogues do not change significantly compared with the dermorphine conformation in aqueous solutions.

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