# HYPOCHROMIC EFFECT AND ELECTRONIC EXCITATION ENERGY TRANSFER IN $Y_t$—$(CH_2)_n$—ADENINE SYSTEMS

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## Summary

Measurements of the UV absorption spectra of aqueous solutions of the bichromophore $Y_t$—$(CH_2)_n$—adenine systems ($n$ = 2, 3, 5 or 6) and the corresponding solutions of 1-methyladenine were carried out. All absorption spectra were subjected to a gaussian component computer analysis and the values of hypochromicity were determined for the individual electronic transitions $S_0 \rightarrow S_n$ in the molecules under investigation. The strongest hypochromic effects were found in systems with $n$ = 3. The relative fluorescence quantum yields of the systems were measured using an equimolar mixture of 1-methyl-$Y_t$ and 9-methyladenine as a reference solution for two different wavenumbers of exciting light: $\tilde{\nu}_1 = 32\,000$ cm$^{-1}$ (where only 1-methyl-$Y_t$ absorbs) and $\tilde{\nu}_2 = 38\,000$ cm$^{-1}$ (where both chromophores absorb). From these measurements, the efficiencies $T$ of the energy transfer from adenine to the $Y_t$ base were determined as 0.41, 0.81, 0.44 and 0.17 for systems with $n$ = 2, 3, 5 or 6 respectively. The results obtained by measuring the hypochromism and the energy transfer are in good agreement.

## 1. Introduction

The $Y_t$ base 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine is the simplest of the modified Y-like bases occurring in transfer ribonucleic acids specific to phenylalanine ($tRNA^{Phe}$). It has been of special interest because of its strong fluorescence which offers a unique tool for probing the conformational properties of $tRNA^{Phe}$ [1]. To study the stacking ability of the $Y_t$ base we have synthesized a series of model compounds in which the $Y_t$ was connected to adenine (its nearest neighbour base in tRNA) by poly-

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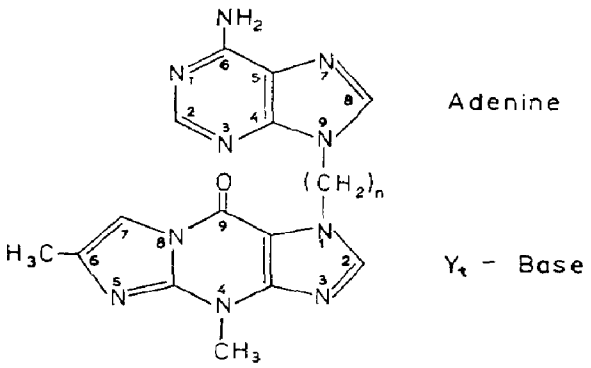


Fig. 1. The structure of the model compounds $Y_t$—$(CH_2)_n$—adenine.

methylene chains of different length, $Y_t$—$(CH_2)_n$—adenine (see Fig. 1) ($n$ = 2, 3, 5 or 6). The absorption and luminescence properties of these compounds have already been studied [2, 3]. The fluorescence quenching experiments reported in refs. 3 and 4 (with α-bromonaphthalene as a quenching substance) allowed the accessibility of the quenching molecules to the $Y_t$ base within these bichromophore systems to be determined. The observation that the fluorescence quenching was least effective in systems with $n$ = 3 was explained by the fact that in this case adenine is closest to the $Y_t$ base, resulting in the greatest screening of the $Y_t$ base from the quenching molecules.

More detailed information concerning the possible conformations and the magnitude of base stacking interactions in these polymethylene analogs of dinucleotides can be obtained from investigations of hypochromic effects and intramolecular electronic excitation energy transfer, and these will be considered in the present paper.

## 2. Materials and methods

The $Y_t$ base was prepared by reacting 3-methylguanine with bromoacetone [5]. The 1-methyl-$Y_t$ was synthesized by direct alkylation of $Y_t$ with methyl iodide in dimethylformamide in the presence of anhydrous $K_2CO_3$. The syntheses and purification of $Y_t$—$(CH_2)_n$—adenine ($n$ = 2, 3, 5 or 6) were carried out as described previously [2].

Absorption spectra were measured on a Beckman Model 25 spectrophotometer. Fluorescence emission measurements were made by using a spectrofluorometer described previously [6]. Corrected fluorescence excitation spectra were recorded using a Perkin–Elmer MPF 44A apparatus equipped with a computer correction unit. All measurements were carried out in a 1 mM sodium cacodylate buffer at pH 7.0. The concentration of the solutions was $5 \times 10^{-5}$ M. A non-linear least-squares fitting method was used for the computer analysis of the absorption spectra, using the well-known Marquardt algorithm [7].

## 3. Results and discussion

### *3.1. Hypochromic effects*

A well-known feature of the UV spectra of nucleic acids is the hypochromic effect which refers to the decreased UV absorption of the polymeric array relative to that of the constituent mononucleotides. The hypochromic effect originates mainly from the coulombic interaction between the dipoles induced by light in nucleic acid bases which are in strong stacking [8 - 10]. The hypochromic effects associated with the base stacking interactions are also observable for some model compounds in which nucleic bases are connected by polymethylene chains, particularly a trimethylene chain ($-(CH_2)_3-$). These compounds generally exhibit conformationally controlled ground state electronic effects similar to those for the dinucleoside phosphates and therefore are very useful models for studying perturbations associated with the 1:1 interaction of the pair of bases [11].

The investigations of the stacking interactions in dinucleotides consisted in measuring the hypochromicity $h$ ($h = 1 - A_{12}/(A_1 + A_2)$ where $A_{12}$, $A_1$ and $A_2$ are the absorbances of the dinucleotide and the respective monomer solutions) which was determined for only one wavelength of the absorbed light, usually 260 nm, or in measuring the hypochromism $H$, usually for the long-wavelength absorption band ($H = 1 - f_{12}/(f_1 + f_2)$ where $f_1$ and $f_2$ are the oscillator strengths of the electronic transition in the free chromophores, $f_{12}$ is the oscillator strength of the electronic transition in the bichromophore system ($f = 4.32 \times 10^{-9} \int \epsilon(\tilde{\nu})\, d\tilde{\nu}$)). It very rarely happens, however, that the absorption band (in the long-wavelength as well as in the short-wavelength region) of a complex organic molecule arises from one electronic transition only. In most cases, the absorption band results from the superposition of different $S_0 \rightarrow S_n$ transitions, the most substantial share being introduced by the $\pi,\pi^*$ transitions, the intensities of which are considerably higher than those of the $n,\pi^*$ bands. Information on the character of the transitions, their energies and intensities can be obtained with increasing accuracy from quantum mechanical calculations, in particular by using spectroscopic parametrization methods. In our opinion the determination of the hypochromism for the individual electronic transitions $S_0 \rightarrow S_n$ is more feasible than determining that for a given absorption band or an interval of the absorption spectrum. In the case of the $Y_t-(CH_2)_n-$adenine systems, the absorption spectrum is the sum of the shares of the individual electronic transitions occurring in adenine and the $Y_t$ base, with the respective weighting functions related to the hypochromic effect taken into account (previous investigations [2] have shown that there are no molecular complexes formed in either ground or excited states for the $Y_t-(CH_2)_n-$adenine systems).

It has been shown by quantum mechanical calculations (CNDO/S and INDO/S) [12, 13] and investigations of the directions of the electronic transition moments [14] that five electronic transitions $\pi,\pi^*$ should be taken into account for the $Y_t$ base in the spectral range 200 - 360 nm. Two strong electronic transitions are observed for aqueous solutions of 9-methyladenine

(I: $\lambda_{max}$ = 261 nm, $\epsilon_{max} = 13.5 \times 10^3$ l mol$^{-1}$ cm$^{-1}$, $f$ = 0.28; II: $\lambda_{max}$ = 206 nm, $\epsilon_{max} = 21 \times 10^3$ l mol$^{-1}$ cm$^{-1}$, $f$ = 0.62) [15, 16].

The absorption spectrum of the $Y_t$—$(CH_2)_n$—adenine systems will thus be the superposition of seven electronic transitions and, according to the above considerations, the intensity changes in each transition should be investigated separately. Hypochromicity in an individual $S_0 \rightarrow S_n$ transition can be defined as

$$h = 1 - \frac{\epsilon_{max}}{\epsilon^0_{max}} \tag{1}$$

where $\epsilon^0_{max}$ and $\epsilon_{max}$ denote the extinction coefficients corresponding to the electronic $S_0 \rightarrow S_n$ transition for a chromophore which is free or bound in the system respectively. Similarly, the hypochromism $H$ can be defined for a given $S_0 \rightarrow S_n$ transition

$$H = 1 - \frac{f}{f^0} = 1 - \frac{\int \epsilon(\tilde{\nu}) d\tilde{\nu}}{\int \epsilon^0(\tilde{\nu}) d\tilde{\nu}} \tag{2}$$

where $f^0$ and $f$ denote the oscillator strengths of the transition $S_0 \rightarrow S_n$ for a chromophore which is free or bound in the system respectively. In order to calculate the value of $h$ and $H$ for each of the electronic transitions, the absorption spectra of the monomer constituents (9-methyladenine and 1-methyl-$Y_t$) and the $Y_t$—$(CH_2)_n$—adenine systems must be analysed as a superposition of bands which correspond to individual transitions. The absorption spectrum was reconstructed from all of these component bands by a computer simulation, in which the band envelope $\epsilon_i(\tilde{\nu})$ of the *ith* component band was assumed to have the conventional form of a gaussian error function at a given wavenumber $\tilde{\nu}$ [17 - 19].

$$\epsilon_i(\tilde{\nu}) = \epsilon_{i,\,max} \exp\left\{ - \frac{(\tilde{\nu} - \tilde{\nu}_{i,\,max})^2}{\delta_i} \right\} \tag{3}$$

where $\tilde{\nu}_{i,\,max}$, $\epsilon_{i,\,max}$ and $\delta_i$ are the peak position, the maximum intensity at $\tilde{\nu}_{i,\,max}$ and the corresponding halfwidth of the *ith* band respectively. The halfwidth is the bandwidth in wavenumbers measured at 1/e of the maximum peak height.

Figures 2 and 3 show the absorption spectra of 1-methyl-$Y_t$ and 9-methyladenine resolved into gaussian bands. Figure 4 shows the absorption spectrum of the $Y_t$—$(CH_2)_n$—adenine system resolved into seven gaussian bands. One can readily decide which of these bands can be attributed to adenine and which belong to the $Y_t$ base. Table 1 summarizes the gaussian parameters of all seven bands for the $Y_t$—$(CH_2)_n$—adenine systems: five bands for 1-methyl-$Y_t$ and two bands for 9-methyladenine. The parameters of each band for the $Y_t$—$(CH_2)_n$—adenine systems should be compared with those for the corresponding bands of 1-methyl-$Y_t$ (bands I, II, IV, V and VII) or 9-methyladenine (bands III and VI). The mean standard deviations for each parameter are given in parentheses. As can be seen, the best fit has been

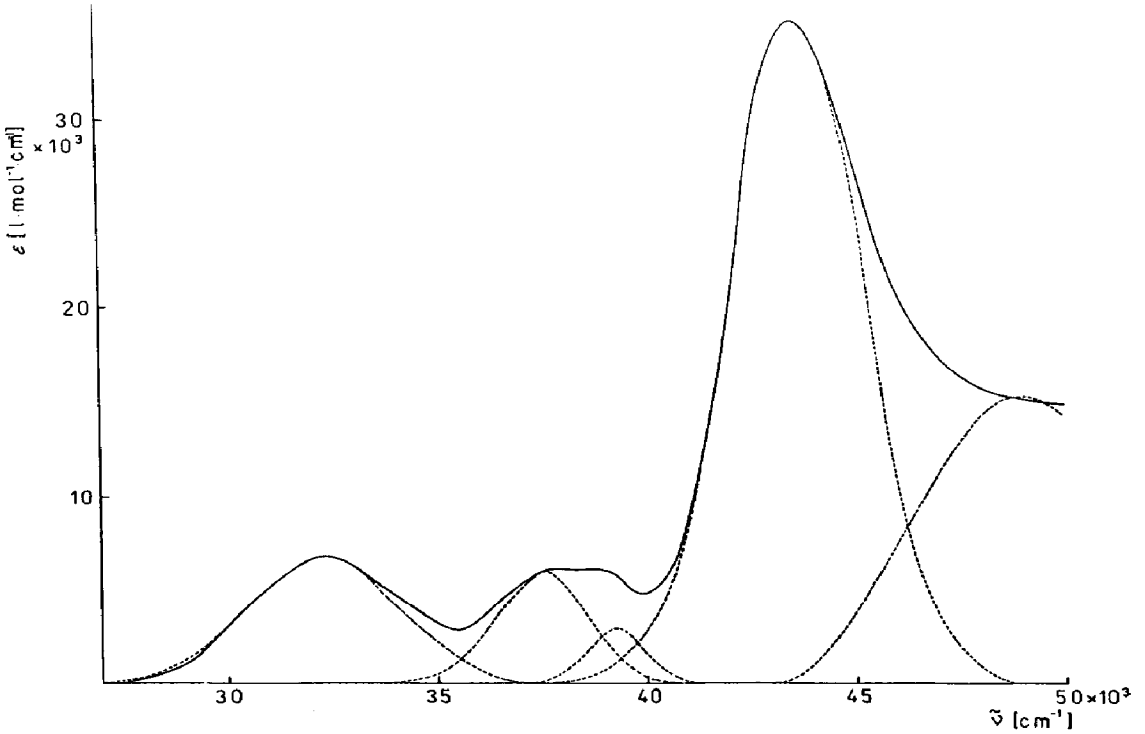


Fig. 2. Absorption spectrum of 1-methyl-$Y_t$ in water at 25 °C.

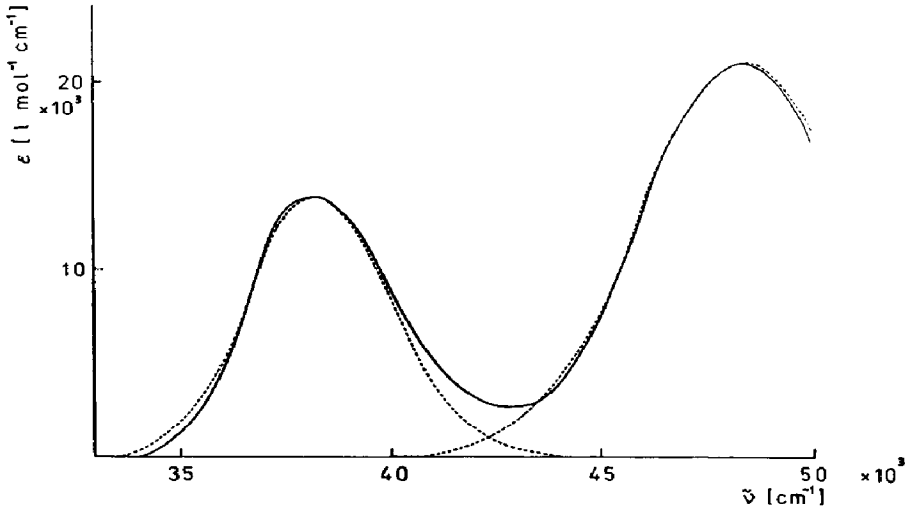


Fig. 3. Absorption spectrum of 9-methyladenine in water at 25 °C.

obtained for band I ($Y_t$), which could have been expected in view of the relatively small overlap of the different bands in this absorption region.

Using the data from Table 1, the hypochromicity *h* and hypochromism *H* values have been calculated for all the individual absorption bands of all the systems studied (see Table 2). In the case of band I ($Y_t$) and the strong

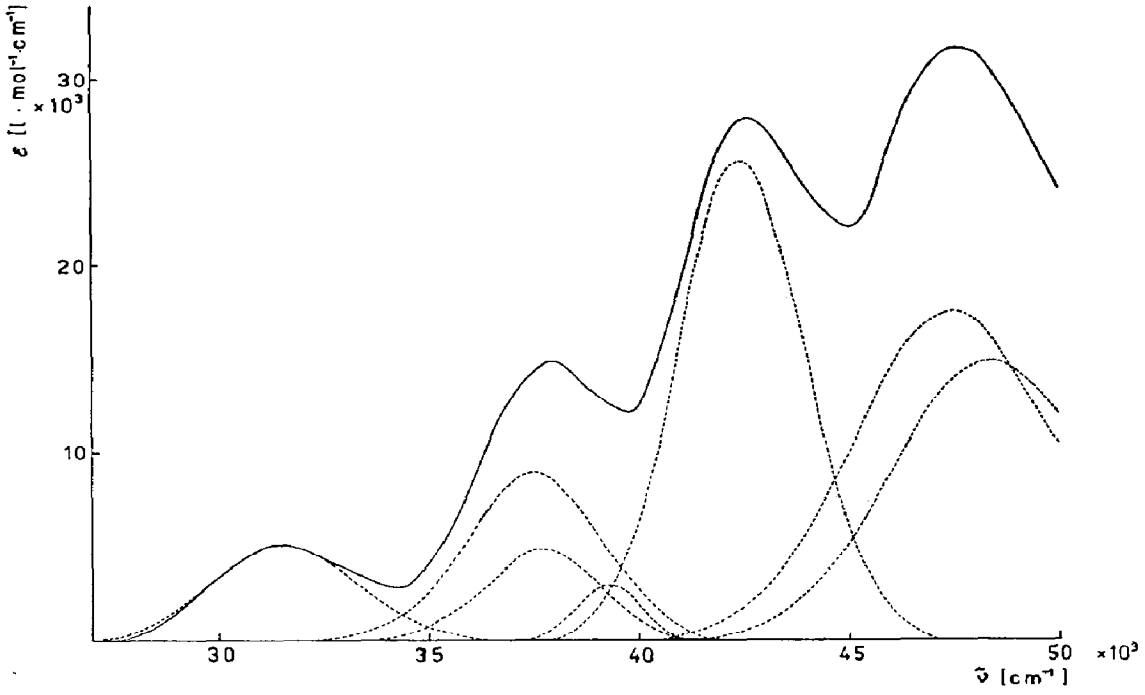


Fig. 4. Absorption spectrum of the $Y_t$—$(CH_2)_3$—adenine system.

bands III (A, adenine), V ($Y_t$), VI (A) and VII ($Y_t$) the standard deviations given in Table 2 are relatively small and do not exceed 5%. The errors for *h* and *H* for the weak IV ($Y_t$) transition are much higher and amount to 25% and 40% for the hypochromicity and hypochromism respectively. The values of hypochromism are in general higher than the respective values of hypochromicity. For the long-wavelength bands I ($Y_t$) and III (A) the effect of the length of the polymethylene chain on the values of the hypochromism *H* on $Y_t$—$(CH_2)_n$—adenine systems is felt in the order $Y_t$—$(CH_2)_3$—adenine > $Y_t$—$(CH_2)_5$—adenine > $Y_t$—$(CH_2)_2$—adenine > $Y_t$—$(CH_2)_6$—adenine. This is in good agreement with the observations for different polymethylene analogs of dinucleotides (see ref. 20 and references cited therein), and reflects the variation in the interaction between the $Y_t$ base and adenine in the systems studied.

Thus, the highest value of hypochromism possessed by the $n = 3$ analog corresponds to an increased degree of base stacking interaction in this system. For bands IV ($Y_t$) and VII ($Y_t$) negative hypochromism effects (hyperchromism) are observed.

However, in the case of the weak transition IV ($Y_t$) this might result from the high standard deviations for the gaussian band parameters (see Table 1). It has already been proved [21] that an optical interaction between the bases could result in either hypochromism or hyperchromism. However, to discuss this observation, a precise knowledge of the relative orientation of the transition moments is needed.

TABLE 1

Parameters of the gaussian bands corresponding to the resolved absorption spectra (mean standard deviations for individual parameters are given in parentheses)

| *Band* | *Parameter* | *1-Methyl-$Y_t$* | *9-Methyl-adenine* | *$Y_t$–$(CH_2)_2$–adenine* | *$Y_t$–$(CH_2)_3$–adenine* | *$Y_t$–$(CH_2)_5$–adenine* | *$Y_t$–$(CH_2)_6$–adenine* |
|---|---|---|---|---|---|---|---|
| I ($Y_t$) | $\epsilon_{max}$ | 6.79 (0.14) | | 5.79 (0.06) | 5.03 (0.05) | 5.58 (0.08) | 5.98 (0.07) |
| | $\tilde{\nu}_{max}$ | 32.34 (0.10) | | 31.89 (0.03) | 31.57 (0.04) | 32.01 (0.04) | 32.03 (0.03) |
| | $\delta$ | 6.81 (0.30) | | 6.03 (0.23) | 5.79 (0.23) | 6.45 (0.31) | 6.37 (0.21) |
| II ($Y_t$) | $\epsilon_{max}$ | 5.75 (0.21) | | 5.60 (0.57) | 4.87 (0.30) | 5.65 (0.41) | 5.18 (0.35) |
| | $\tilde{\nu}_{max}$ | 37.53 (0.26) | | 37.42 (0.19) | 37.68 (0.57) | 37.18 (0.20) | 37.07 (0.08) |
| | $\delta$ | 2.66 (0.36) | | 2.66 (0.40) | 3.26 (0.42) | 2.47 (0.24) | 2.64 (0.14) |
| III (A) | $\epsilon_{max}$ | | 13.65 (0.34) | 10.97 (0.65) | 8.93 (0.80) | 10.21 (0.66) | 13.18 (0.31) |
| | $\tilde{\nu}_{max}$ | | 38.35 (0.08) | 38.13 (0.19) | 37.48 (0.38) | 38.07 (0.11) | 38.30 (0.09) |
| | $\delta$ | | 5.61 (0.54) | 5.46 (0.31) | 5.32 (0.91) | 5.30 (0.39) | 5.52 (0.27) |
| IV ($Y_t$) | $\epsilon_{max}$ | 2.87 (0.36) | | 2.97 (0.32) | 2.93 (0.71) | 2.57 (0.37) | 2.32 (0.26) |
| | $\tilde{\nu}_{max}$ | 39.24 (0.25) | | 39.45 (0.08) | 39.30 (0.16) | 39.41 (0.09) | 39.45 (0.05) |
| | $\delta$ | 0.98 (0.36) | | 1.32 (0.12) | 1.36 (0.15) | 1.18 (0.10) | 0.99 (0.09) |
| V ($Y_t$) | $\epsilon_{max}$ | 35.10 (0.32) | | 29.44 (1.17) | 25.63 (1.34) | 30.18 (1.23) | 31.29 (0.98) |
| | $\tilde{\nu}_{max}$ | 43.55 (0.03) | | 42.87 (0.04) | 42.61 (0.07) | 42.79 (0.08) | 43.12 (0.10) |
| | $\delta$ | 4.76 (0.12) | | 4.62 (0.16) | 4.46 (0.20) | 4.53 (0.31) | 4.65 (0.18) |
| VI (A) | $\epsilon_{max}$ | | 20.80 (0.39) | 17.94 (0.63) | 17.48 (0.72) | 18.20 (0.66) | 20.16 (0.74) |
| | $\tilde{\nu}_{max}$ | | 48.31 (0.11) | 48.01 (0.22) | 47.56 (0.21) | 47.98 (0.26) | 48.10 (0.16) |
| | $\delta$ | | 12.01 (0.61) | 10.22 (0.83) | 10.94 (0.81) | 10.44 (0.79) | 11.48 (0.81) |
| VII ($Y_t$) | $\epsilon_{max}$ | 15.16 (0.41) | | 16.40 (0.97) | 14.81 (1.02) | 17.34 (1.01) | 15.61 (1.06) |
| | $\tilde{\nu}_{max}$ | 48.96 (0.12) | | 48.72 (0.29) | 48.41 (0.27) | 48.86 (0.30) | 48.81 (0.22) |
| | $\delta$ | 10.88 (0.46) | | 11.18 (0.77) | 11.12 (0.86) | 11.22 (0.84) | 11.22 (0.90) |

Units: $\epsilon_{max}$, l mol$^{-1}$ cm$^{-1}$ x $10^3$; $\tilde{\nu}_{max}$, cm$^{-1}$ x $10^3$; $\delta$, cm$^{-2}$ x $10^3$.

TABLE 2

Hypochromicity $h$ and hypochromism $H$ for the individual electronic transitions of the $Y_t-(CH_2)_n-$adenine systems

| Band | $Y_t-(CH_2)_2-$adenine | | $Y_t-(CH_2)_3-$adenine | | $Y_t-(CH_2)_5-$adenine | | $Y_t-(CH_2)_6-$adenine | |
|---|---|---|---|---|---|---|---|---|
| | $h$ | $H$ | $h$ | $H$ | $h$ | $H$ | $h$ | $H$ |
| I ($Y_t$) | 0.148 | 0.198 | 0.259 | 0.317 | 0.178 | 0.200 | 0.120 | 0.148 |
| II ($Y_t$) | 0.026 | 0.026 | 0.153 | 0.062 | 0.017 | 0.053 | 0.099 | 0.103 |
| III (A) | 0.196 | 0.207 | 0.346 | 0.363 | 0.252 | 0.273 | 0.034 | 0.042 |
| IV ($Y_t$) | −0.035 | −0.201 | −0.021 | −0.203 | 0.105 | 0.017 | 0.192 | 0.188 |
| V ($Y_t$) | 0.161 | 0.174 | 0.270 | 0.293 | 0.140 | 0.161 | 0.109 | 0.119 |
| VI (A) | 0.137 | 0.204 | 0.160 | 0.198 | 0.125 | 0.184 | 0.031 | 0.052 |
| VII ($Y_t$) | −0.082 | −0.097 | 0.023 | 0.012 | −0.144 | −0.162 | −0.030 | −0.093 |

*3.2. Electronic excitation energy transfer in the $Y_t$—$(CH_2)_n$—adenine systems*

It has already been proved that stacking interaction in dinucleotides or related bichromophoric systems can also be studied by means of energy transfer measurements [22, 23]. Evidence for intramolecular energy transfer in such systems can usually be obtained from a comparison of corrected fluorescence excitation spectra with absorption spectra.

The absorption and corrected excitation spectra for $Y_t$—$(CH_2)_n$—adenine systems ($n$ = 2, 3 or 6) are shown in Fig. 5 (the corresponding spectra for the $n$ = 5 analog are very similar to those of $Y_t$—$(CH_2)_2$—adenine and are not shown here).

It is possible to compare these spectra by normalizing them to the same intensity at the long-wavelength maximum (where only $Y_t$ absorbs) for each analog. The comparison of the intensities of the excitation and absorption curves at the short-wavelength part of the spectra ($\tilde{\nu}$ = 38 000 $cm^{-1}$), where both adenine and $Y_t$ chromophores absorb, provides direct evidence for

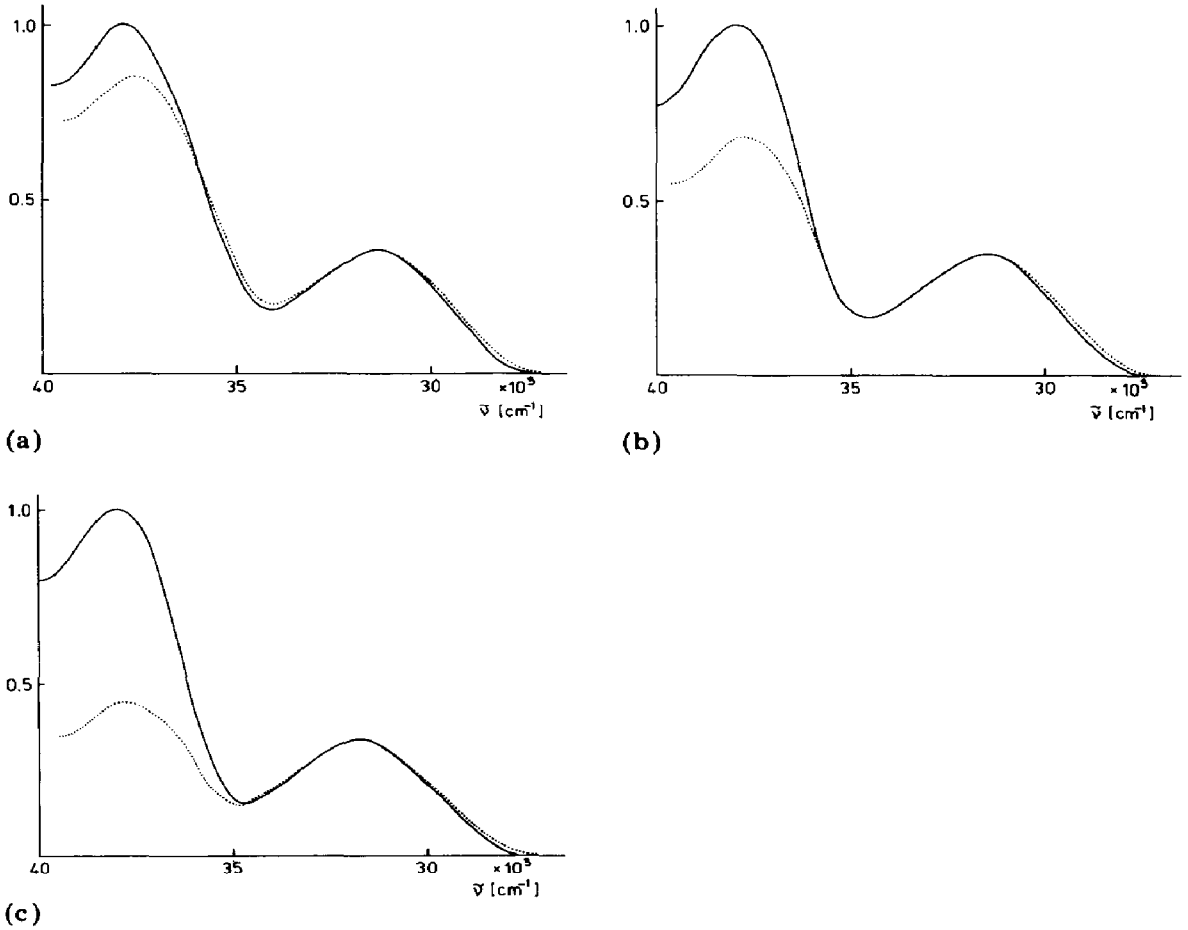


Fig. 5. Comparison of the absorption (———) and corrected (·····) fluorescence excitation spectra of (a) $Y_t$—$(CH_2)_3$—adenine, (b) $Y_t$—$(CH_2)_5$—adenine and (c) $Y_t$— $(CH_2)_6$—adenine.

singlet energy transfer from adenine to the $Y_t$ base within these systems. It is clear that the energy transfer process is strongly influenced by the length of the polymethylene chain and is most efficient for the $n = 3$ analog.

A quantitative determination of the efficiency of the energy transfer can be obtained by measuring the fluorescence quantum yield of the system investigated and that of an equimolar mixture of 9-methyladenine and 1-methyl-$Y_t$ at two different wavenumbers of the exciting light, *i.e.* in the region where only the acceptor absorbs ($\tilde{\nu}_1 = 32\,000$ cm$^{-1}$), and where both donor and acceptor molecules absorb ($\tilde{\nu}_2 = 38\,000$ cm$^{-1}$). In general, for an equimolar mixture of the donor and acceptor molecules, the quantum yield on short-wavelength excitation can be written as

$$\varphi_0 = \alpha_D{}^0\varphi_D{}^0 + \alpha_A{}^0\varphi_A{}^0 \tag{4}$$

and for the whole system

$$\varphi = \alpha_D\varphi_D(1 - T) + \alpha_A\varphi_A + \alpha_D T\varphi_A \tag{5}$$

where $\alpha_D{}^0$, $\alpha_A{}^0$ and $\alpha_D$, $\alpha_A$ are the light fractions absorbed by the donor and acceptor in the equimolar mixture and in the system respectively, $\varphi_D{}^0$, $\varphi_A{}^0$ and $\varphi_D$, $\varphi_A$ are the fluorescence quantum yields for free and bound chromophore moieties respectively and $\varphi_0$ and $\varphi$ are the fluorescence quantum yields of the solution of the equimolar mixture and the system respectively:

$$\frac{\varphi}{\varphi_0} = \frac{\int I(\tilde{\nu})\mathrm{d}\tilde{\nu}}{\int I_0(\tilde{\nu})\mathrm{d}\tilde{\nu}}$$

For the sensitized acceptor fluorescence, the second term in eqn. (4) and the second and third terms in eqn. (5) should be considered:

$$T = \frac{\varphi}{\varphi_0}\frac{\varphi_A{}^0}{\varphi_A}\frac{\alpha_A{}^0}{\alpha_D} - \frac{\alpha_A}{\alpha_D} \tag{6}$$

In aqueous solutions adenine has an insignificantly low fluorescence quantum yield, and therefore the transfer efficiency $T$ can only be determined from the sensitized fluorescence of the $Y_t$ base, *i.e.* using eqn. (6).

The values of the ratios $\varphi_A{}^0/\varphi_A$ can be found by exciting the mixture of chromophores and the $Y_t$—$(CH_2)_n$—adenine systems in the long-wavelength spectral region ($\tilde{\nu}_1 = 32\,000$ cm$^{-1}$). Under fixed experimental conditions and $\tilde{\nu}_{exc} = 32\,000$ cm$^{-1}$

$$\frac{\varphi_A{}^0}{\varphi_A} = \frac{\int I_A{}^0(\tilde{\nu})\mathrm{d}\tilde{\nu}}{\int I_A(\tilde{\nu})\mathrm{d}\tilde{\nu}}\,\frac{1 - 10^{-A_A}}{1 - 10^{-A_A{}^0}} \tag{7}$$

where $A_A$ and $A_A{}^0$ are the absorbances of the systems and the equimolar mixture of monomer solutions respectively, determined for $\tilde{\nu} = 32\,000$ cm$^{-1}$. The ratios $\varphi/\varphi_0$ are obtained by exciting the systems and the mixture in the short-wavelength region ($\tilde{\nu}_2 = 38\,000$ cm$^{-1}$). The ratios $\alpha_A/\alpha_D$ and $\alpha_A{}^0/\alpha_D$ for this excitation can be determined (for low absorbances) from the absorption law

TABLE 3

Parameters relating to the intramolecular singlet energy transfer in the $Y_t-(CH_2)_n-$adenine systems (the errors estimated for the parameter $T$ are given in parentheses)

| *Compound* | $\varphi/\varphi_0$ | $\varphi_A/\varphi_A{}^0$ | $T$ (%) |
|---|---|---|---|
| $Y_t-(CH_2)_2-$adenine | 2.48 | 1.22 | 41 (10) |
| $Y_t-(CH_2)_3-$adenine | 2.63 | 0.97 | 81 (15) |
| $Y_t-(CH_2)_5-$adenine | 3.44 | 1.59 | 44 (10) |
| $Y_t-(CH_2)_6-$adenine | 2.12 | 1.38 | 17 (10) |

$$\frac{\alpha_A}{\alpha_D} = \frac{A_A}{A_D} \tag{8}$$

and

$$\frac{\alpha_A{}^0}{\alpha_D} = \frac{1 - 10^{-(A_A{}^0 + A_D{}^0)}}{1 - 10^{-(A_A + A_D)}} \frac{1 + A_A/A_D}{1 + A_D{}^0/A_A{}^0} \tag{9}$$

where $A_A$, $A_D$ and $A_A{}^0$, $A_D{}^0$ are the absorbances at $\tilde{\nu}_2 = 38\,000$ cm$^{-1}$ of the acceptor and donor in the systems and a reference mixture respectively.

$A_A$ and $A_D$ values were determined from the resolved spectra of the systems (see Fig. 4 and Table 2) and were thus corrected for the hypochromic effects. The values of the energy transfer efficiencies $T$, derived from the above formulae, are shown in Table 3. (The calculated errors result mainly from the relatively high standard deviations for the $A_A$ and $A_D$ parameters.) The results obtained are very consistent with the corrected observed excitation spectra and correlate very well with the calculated hypochromic effects. The highest value of $T$, for the $n = 3$ analog, can be explained by the existence of a small separation between the chromophores which is achieved by strong "stacking" in this system. The application of the Förster formula [24] for a critical radius $R_0$ for dipole–dipole interaction gave for the adenine (donor) and $Y_t$ base (acceptor) system a value of $R_0 = 4$ Å. Such a low value of $R_0$ results mainly from the very low quantum yield of the donor (adenine) fluorescence ($3 \times 10^{-6}$ according to ref. 25). Although the assumption that only dipole–dipole interactions are important at such small distances is certainly an approximation, the values $R$ for the donor–acceptor separations estimated using eqn. (10) ($R = 3$ Å for $n = 3$, $R = 4.2$ Å for $n = 2$ and $n = 5$, and $R = 5.2$ Å for $n = 6$) would seem to be reasonable if the structure is considered (see Fig. 1) and a different degree of stacking is assumed for each analog:

$$R = R_0\left(\frac{1}{T} - 1\right)^{1/6} \tag{10}$$

It has already been proved that even at distances of 4 - 5 Å the dipole–dipole interaction predominates [26]. Thus it is very probable that this mechanism has the largest contribution for the analogs with $n = 2$, 5 or 6. In

the system, however, with $n = 3$ a substantial exchange interaction must be considered [27].

## 4. Conclusions

The investigations of the hypochromic effects, as well as the intramolecular electronic excitation energy transfer, indicate that the system $Y_t$—$(CH_2)_3$—adenine in aqueous solution is in a stacked conformation. A small separation of the chromophores leads to strong hypochromic effects for individual electronic transitions and to high energy transfer efficiency, but does not result in the formation of complexes in the ground or excited states. The elongation of the polymethylene bridge results in a decrease in the hypochromic effect and the energy transfer efficiency, which indicates inefficient stacking interactions within these compounds.

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