Energy Transfer in Modified DNA

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Communicated by Martin D. Kamen, November 4, 1970

ABSTRACT Energy transfer at room temperature and in aqueous solution between two hydrocarbon chromophores, 2-acetylaminofluorene and 7-methylbenz(a)anthracene, covalently attached to DNA and to homopoly- nucleotide models is described. Transfer, as evidenced by changes in fluorescence intensity and polarization, is a function of secondary structure.

Study of energy transfer in polynucleotides at room temperature in aqueous solution at pH 7 is inhibited by the lack of fluorescence or phosphorescence of the major natural nucleotides under these conditions. No evidence of transfer could be obtained even when synthetic polynucleotides and complexes containing rare (but natural) bases such as N*-acetylcytidine, 7-methylinosine, or 7-methylguanosine which fluoresce in aqueous solution, albeit with a low quantum yield, were examined in aqueous solution (1). Although energy transfer has indeed been demonstrated in nucleic acids in a rigid medium at 77°K, it is difficult to extrapolate such results to aqueous conditions (2). Intercalation of acridine derivatives in DNA gives rise to an energy transfer from the nucleotide residue absorbing light to the dye, though demonstrations of transfer via several bases is much less clear (3). Other studies have shown a triplet transfer from photosensitizers such as acetonaphone to thymine residues (4). Energy transfer from the fluorescent Y base in yeast tRNA^Phe to dye stuffs covalently bound to the oxidized ribose residue of the 3'-terminal adenosine has also been demonstrated (8).

The present report describes energy transfer at long distance (15–20 Å) between hydrocarbon chromophores covalently linked to bases in synthetic polynucleotides and DNA in aqueous solution at room temperature. Two different chromophores, 7-methylbenz(a)anthracene and 2-acetylamino- fluorene, with completely different fluorescence characteristics were employed.

MATERIAL AND METHODS
UV absorption spectra were recorded with a Cary 15 spectrophotometer. Fluorescence spectra were obtained with a double monochromator Zeiss fluorimeter. All excitation and emission spectra were corrected. Values of polarization were determined with a fluorescence polarization spectrophotometer equipped with two photomultipliers to detect separately each polarized component of the fluorescent light (5). The source was a 450 W Xenon lamp. All emissions were measured above 415 nm with the aid of MTO cutoff filters. Concentrations used for fluorescence spectra or fluorescence polarization values were 0.1 μmol/ml in mononucleotides or less. Fluorescence lifetimes were measured with a TRW model 75 A decay-time fluorimeter with a 31B nanosecond spectral source and a type 556 Tektronix dual-beam oscilloscope. Optical rotatory dispersion spectra were obtained with a Spectropol 1 (Fica).

7-Methylbenz(a)anthracene (MBA) derivative (2.6% substitution) of polyguanylic acid
To a solution of 5 mg of poly(G) in a 1:1 water–propyleneglycol mixture (2 ml) at 20°C was added 5 mg of 7-bromomethylbenz(a)anthracene in acetone (1 ml). After 10 min, the solution was extracted 3 times with 2 ml of ether (extracts were discarded) and the aqueous solution was dialyzed overnight against water and then lyophilized.

MBA derivative (1% substitution) of polyadenylic acid
To a solution of 10 mg of poly(A) in 1:1 water–propyleneglycol mixture (6 ml) at 20°C was added 5 mg of 7-bromomethylbenz(a)anthracene in 1 ml of acetone. After one extraction with ether, the remaining poly(A) was treated again with the carcinogen under the same conditions. After 10 min, the solution was extracted thrice with 2 ml of ether and the aqueous solution was dialyzed overnight against water and lyophilized.

MBA derivative (6–8% substitution) of DNA
To a solution of Micrococcus lysodeikticus DNA (5 mg) in 3 ml of 0.005 M sodium cacodylate buffer (pH 7.0)–0.01 M NaCl were added 3 ml of propyleneglycol and 1 mg of 7-bromomethylbenz(a)anthracene. After 10 min at 20°C the solution was extracted 3 times with ether, the extracts were discarded, and 1.5 ml of 4 M NaCl was added to the aqueous solution. The DNA was then precipitated by the addition of 12 ml of ethanol.

In each case, the quantity of methylbenz(a)anthracene covalently bound to the polynucleotide was estimated by comparison of the absorption at 350 and 260 nm after acid hydrolysis of the DNA (1 M HCl at 100°C for 1 hr) or alkaline hydrolysis (0.2 M NaOH at 20°C for 24 hr) for the polyribonucleotides. For reasons of solubility, the hydrolysates were dissolved in 50% ethanol for the measurement of UV absorbances. Molar extinction coefficients (ε) of 10,000 for the nucleotide residue (at 260 nm) and of 10,000 and 50,000 for the hydrocarbon residue (at 350 and 260 nm) in the monomer conjugates were used.

Abbreviations: AAF, 2-acetylaminofluorene; MBA, 7-methylbenz(a)anthracene.
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was estimated by assuming an \( \epsilon_{302} \) equal to 20,000 and an absorbance ratio 302 nm/256 nm of 1.2.

**Poly(A)-MBA(1%)-AAF(10.4%)**

To 1 mg of poly(A)-MBA(1%) in 0.1 ml of water was added 5 \( \mu \)mol of N-acetoxy AAF in 1.5 ml of propylene glycol, and the solution was kept at 37°C for 3 hr. After addition of 2:1 water-propylene glycol (4 ml), the mixture was extracted 3 times with ether and dialyzed extensively against water and then against 0.01 M NaCl-0.005 M sodium cacodylate buffer, pH 7.0.

**M. lysodeikticus DNA-MBA(6%)-AAF(34%)**

To 3 \( \mu \)mol of DNA-MBA(6%) in 1 ml of 0.01 M NaCl-0.005 M sodium cacodylate, pH 7.0 was added 3.3 \( \mu \)mol of N-acetoxy AAF in propylene glycol. The solution was incubated at 37°C for 3 hr, extracted with ether, and dialyzed against 0.01 M NaCl-0.005 M sodium cacodylate, pH 7.0. Other preparations of DNA, containing different proportions of AAF, were prepared in the same way by addition of appropriate amounts of N-acetoxy AAF.

**RESULTS AND DISCUSSION**

Both N-acetoxy AAF (6) and 7-bromo MBA (7) react with poly(G) at position 8 of the guanine residues (data to be published). The two carcinogens also react with adenine residues in poly(A), though the nature of the product is not yet completely defined. Under the conditions used, no reaction occurs with polycytidylic acid or polypuridylic acid. As Fig. 1 shows, there is considerable overlap between 200 and 320 nm in the absorption spectra of the monomer products GMP-AAF and GMP-MBA, the latter showing absorption at longer wavelengths.

**Treatment of poly(G) (2.6% substituted with MBA) with N-acetoxy-2-acetyl aminofluorene**

Three equal portions of poly(G)-MBA(2.6%) each containing 0.5 \( \mu \)mol of nucleotide were treated with 0.5, 1.5, and 3.0 \( \mu \)mol of N-acetoxy AAF, at room temperature for 2 hr in 1:1 aqueous propylene glycol. The reaction mixtures were extracted 3 times with 2-ml portions of ether and the aqueous solutions were dialyzed extensively against water.

An aliquot of each was hydrolyzed in 0.1 M KOH for 30 min at 80°C and the substitution by 2-acetylamino-fluorene was estimated from the spectra of the alkaline hydrolysate. The results showed 6.8, 9.6, and 11.6% substitution, respectively.

**Poly(A) substituted 1% with MBA and 2.5% with AAF**

To a solution of 0.7 mg of poly(A)-MBA(1%) in 0.4 ml water was added 4 \( \mu \)mol of N-acetoxy AAF in 0.4 ml of propylene glycol. After incubation at 37°C for 3 hr, the solution was extracted 3 times with ether and then dialyzed extensively against water and finally against 0.01 M sodium cacodylate, pH 7.0. The amount of AAF covalently bound to the poly(A) was estimated by assuming an \( \epsilon_{302} \) equal to 20,000 and an absorbance ratio 302 nm/256 nm of 1.2.
wavelengths up to 400 nm. In the wavelength band 300–310 nm the absorption coefficient of GMP-AAF, which shows very little or no fluorescence, is approximately twice that of the highly fluorescent MBA-MBA.

Energy transfer from donor fluoresce residues to benzan-thracene acceptors in poly(G) that is conjugated with both hydrocarbons is indicated in Figs. 2 and 3 which show the fluorescence excitation spectra and difference spectra of samples of poly(G) all containing 2.6% residues substituted by MBA and with 0, 6.8, 9.6, and 11.6% substitution by AAF. An increase of up to 40% in the intensity of fluorescence (I_F) by excitation at 300 nm may be noted. Excitation spectra were normalized at 360 nm to eliminate possible variations of I_F due to conformational changes. At this wavelength the donor residues do not absorb and fluorescence is due only to MBA residues in the absence of energy transfer.

A rough approximation gives an average of one to two donor residues (AAF) for 1 turn of a double helix, with a receptor (MBA) every 2 turns of the helix. Although distribution of the hydrocarbon residues is probably roughly random, this suggests transfer at distances of 15–20 Å. Transfer by collision between markers on different polynucleotide chains appears unlikely at nucleotide concentrations (in terms of monomer) of 10^{-4} M or less. It may be noted that even though up to 15% of the guanine residues are substituted by hydrophobic, sterically hindering groups, the secondary structure of the poly(G) is not significantly modified, as shown by the essentially unchanged optical rotatory dispersion curves of the different copolymers.

The differently substituted poly(G) all give double-stranded complexes with poly(C). Fluorescence excitation spectra (Fig. 3B and 4) of these complexes also show an increased intensity of fluorescence by excitation in the region where GMP-AAF residues absorb. The spectra show poorer resolution at 280–295 nm compared with the results described above, probably because of the much larger absorption by poly(C) at these wavelengths compared to poly(G).

Evidence of energy transfer is also seen in the variations of fluorescence polarization for the different polymers and complexes (Table 1). These variations are maximal at 310 nm, at which wavelength transfer is most efficient, as shown by the excitation spectra. Increase in percentage of donor molecules (AAF) in the polymers and complexes increases the depolarization. The percentage depolarization is less for the complexes with poly(C) (32–40%) than for the modified poly(G).

![Figure 4](image1.png)

**Fig. 4.** Excitation and emission spectra of poly(G)-MBA (2.6%) -- , poly(G)-MBA (6.8%) -- , and the same complexes containing 9.6% AAF, and 11.6% AAF --- in the poly(G) strand. Excitation spectra at 310 nm are normalized at 360 nm. Excitation at 310 nm for emission spectra. All solutions in 0.01 M NaCl-0.005 M sodium cacodylate, pH 7.0 at 20°C. Fluorescence in arbitrary units.

![Figure 5](image2.png)

**Fig. 5.** Excitation and emission spectra of poly(A)-MBA- (1%)-- , poly(A)-MBA (1%)-AAF (2.5%) -- , poly(A)-MBA (1%)-AAF (5%) -- , and the same complex with 10.4% AAF ---. Excitation spectra (emission 420 nm) are normalized at 360 nm and emission spectra at 420 nm (excitation 310 nm). All solutions in 0.01 M NaCl-0.005 M sodium cacodylate, pH 7.0 at 20°C. Fluorescence in arbitrary units.

<p>| Table 1. Fluorescence polarization* of conjugates of MBA (2.6%) and AAF with poly(G). |
|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>( \lambda ) (nm)</th>
<th>poly(G)-MBA (2.6%)</th>
<th>poly(G)-MBA (6.8%)</th>
<th>poly(G)-MBA (9.6%)</th>
<th>poly(G)-MBA (11.6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>305</td>
<td>0.040</td>
<td>0.028</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>310</td>
<td>0.048</td>
<td>0.027</td>
<td>0.024</td>
<td>0.025</td>
</tr>
<tr>
<td>315</td>
<td>0.048</td>
<td>0.030</td>
<td>0.026</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Excitation at 315 nm</strong></td>
<td>( \tau = 24 \text{nsec} )</td>
<td>( \tau = 21 \text{nsec} )</td>
<td>( \tau = 20 \text{nsec} )</td>
<td>( \tau = 20 \text{nsec} )</td>
</tr>
<tr>
<td>+ poly(C)</td>
<td>+ poly(C)</td>
<td>+ poly(C)</td>
<td>+ poly(C)</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>0.044</td>
<td>0.034</td>
<td>0.027</td>
<td>0.027</td>
</tr>
<tr>
<td>310</td>
<td>0.047</td>
<td>0.032</td>
<td>0.029</td>
<td>0.028</td>
</tr>
<tr>
<td>315</td>
<td>0.047</td>
<td>0.032</td>
<td>0.032</td>
<td>0.030</td>
</tr>
</tbody>
</table>

* Values are normalized at 370 nm with respect to poly(G)-MBA (2.6%) or with the same conjugate complexed with poly(C). Solutions are in water for poly(G) conjugates and in 0.01 M NaCl-0.005 M sodium cacodylate (pH 7.0) for conjugates complexed with poly(C).
alone (44–50%), in accord with a lower efficiency of transfer in the former case as suggested by the excitation spectra. However, correlation of transfer with structure and conformation is much clearer in the case of modified poly(A) and complexes with poly(U).

The fluorescence characteristics of poly(A) with 1% of the bases conjugated with MBA and 2.5% with AAF (polynucleotide in random-coil form) show no signs of energy transfer. However, when the polymer is complexed with poly(U) [melting temperature of the complex is 2°C higher than that of poly(A)-poly(U)], transfer occurs, as shown in Fig. 5.

**TABLE 2. Fluorescence polarization of Micrococcus lysodeikticus DNA-MBA(6%) - AAF conjugates**

<table>
<thead>
<tr>
<th>DNA-MBA(6%)</th>
<th>DNA-MBA(6%) - AAF(34%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda ) (nm)</td>
<td>Buffer A ( \dagger )</td>
</tr>
<tr>
<td>305</td>
<td>0.043</td>
</tr>
<tr>
<td>310</td>
<td>0.042</td>
</tr>
<tr>
<td>315</td>
<td>0.037</td>
</tr>
<tr>
<td>( T_m ) (260°C)</td>
<td>84.5°C</td>
</tr>
</tbody>
</table>

* Values are normalized at 360 nm with respect to DNA-MBA(6%).

† Buffer A, 0.01 M NaCl-0.005M sodium cacodylate, pH 7.0.

‡ \( T_m \), melting temperature.

Increasing the percentage of AAF donor residues to 10.4% (the acceptor remaining constant) quenches fluorescence in the single-stranded poly(A), while the complex with poly(U) shows essentially the same excitation spectrum as the control poly(A)-MBA(1%)-poly(U), that is, transfer is abolished. However, with 10.4% AAF the melting temperature of the complex with poly(U) is lowered (by about 3°C) and it is likely that secondary structure in the region of the modifications is changed, such that the relative orientations of the transition dipoles of the two hydrocarbons are disordered, thus decreasing the efficiency of energy transfer (9, 10).

Variation of the efficiency of energy transfer as a function of secondary structure is also noted with modified DNA (from *M. lysodeikticus*). The melting temperature of DNA containing 6% MBA is some 3°C higher than that of the original DNA. (Preliminary examination suggests the presence of cross-linkages.) Further substitution by the aminofluorene (34%) reduces the melting temperature (Fig. 6A), though the "native" form of the DNA is maintained. This is also indicated by the optical rotatory dispersion spectra (Fig. 6B).

Acid hydrolysis of the modified DNA (1 N HCl at 80°C for 1 hr) followed by chromatographic separation of the products showed that equal quantities of adenine and guanine residues were substituted.

Fluorescence excitation spectra at 20°C of the DNA-MBA-AAF(34%) show a 3-fold increase in intensity at 310 nm compared with the control DNA substituted solely with MBA (Fig. 7A). In contrast, the acid hydrolysate containing monomer residues shows a relative diminution in fluorescence (Fig. 7B), after correction for the fluorescence of the hydrolysate product of adenine conjugated with the acetylaminofluorene.
TABLE 3. Fluorescence polarization of M. lysodeikticus DNA-MBA(8%)-AAF conjugates*

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>0</th>
<th>10</th>
<th>25</th>
<th>37</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>310</td>
<td>0.037</td>
<td>0.033</td>
<td>0.025</td>
<td>0.032</td>
<td>0.030</td>
</tr>
<tr>
<td>315</td>
<td>0.038</td>
<td>0.029</td>
<td>0.024</td>
<td>0.026</td>
<td>0.028</td>
</tr>
<tr>
<td>Tm (260 nm)</td>
<td>67.5°C</td>
<td>62°C</td>
<td>60°C</td>
<td>59°C</td>
<td>57°C</td>
</tr>
</tbody>
</table>

* All solutions in 0.005 M NaCl-0.0025 M cacodylate, pH 7.0 in 1:1 water–ethanol. These values are normalized at 360 nm with respect to DNA-MBA(8%).

Enzymatic hydrolysis (pancreatic DNase and venom phosphodiesterase) gives high molecular weight products in which energy transfer can be demonstrated, although it is somewhat diminished.

At 20°C, the fluorescence excitation spectra of the doubly substituted DNA in aqueous salt solution or in 1:1 water–ethanol are identical. At 90°C the strands are only partially separated (because of cross-linking) in the first solvent and energy transfer is still visible, but this is completely suppressed in the ethanol solution. Ultraviolet absorption spectra of DNA-MBA(6%) in aqueous and aqueous ethanol solution at 20 and 90°C (Fig. 8) suggest that in aqueous solution the orientations of the transition dipoles of hydrocarbon molecules do not vary (thermal denaturation gives 12% hypochromicity and a blue shift of 2 nm in the visible region) while in ethanol solution, this orientation, relative to the helical axis, is disturbed (thermal hypochromicity of 24%, with a blue shift of 5 nm). Acid hydrolysis gives a 50% increase in visible absorption and a 6 nm blue shift.

As with poly(G), energy transfer is also characterized by changes in fluorescence polarization in the region 305-315 nm (Table 2). At 315 nm, there is 48% depolarization in aqueous solution compared with 38% in the presence of ethanol. This lowered efficiency of transfer is also seen in the fluorescence excitation spectra. A similar parallelism between depolarization and energy transfer as indicated by increased intensity of fluorescence is shown in the properties of a series of modified DNAs in which the quantity of acceptor (MBA) is constant but the percentage of donor (AAF) residues varies. Thus for four preparations of M. lysodeikticus DNA, all containing 8% of the nucleotides conjugated to MBA, but with 10, 25, 37, and 42% of the residues linked to AAF, the maximum increase in intensity of the fluorescence excitation spectra was noted at 25% substitution by the donor, coincident (Table 3) with maximum depolarization (24, 37, 31 and 26% at 315 nm respectively). Since the ratio of fluorescence intensity by excitation at 310 nm of DNA-MBA(6%) relative to that of DNA-MBA(6%)-AAF(20%) is independent of viscosity over a range of 1.7 to 49 centipoises (sucrose solutions); it is unlikely that transfer by contact between distinct polymer molecules plays an important role in the transfer of energy described above. In any case, at high levels of substitution with most of the purine bases modified, direct transfer between hydrocarbon residues at contact distance in the double helix must be much more significant.

This work was supported by grant no. 6600206 from the Délégation Générale à la Recherche Scientifique et Technique.