LOW TEMPERATURE EMISSION SPECTRA OF POLY(G), POLY(G).POLY(C), AND POLY(G,C)*

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The luminescence spectra of poly(G), poly(G).poly(C), and poly(G,C) at neutral pH have been measured in different solvents at 77 K. The fluorescence maxima of poly(G) and poly(G).poly(C) are shifted to lower energies as compared with the spectrum of monomeric GMP, and correspond to the emission from excimer states. The excimer peak of poly(G).poly(C) is red-shifted relatively to that of either poly(G) or poly(C). The singlet emission of poly(G,C) is identical with that of the dinucleotide CpG and consists of two peaks: The low energy one corresponds to the excimer emission, the other one to the emission from the non-interacting residues. The phosphorescence spectra of the three polynucleotides are similar to the spectrum of GMP, but slightly red shifted. The phosphorescence decay is non-exponential. Besides the component characteristic of the guanine residues (1·4—1·5 s) it contains a short-lived component, which corresponds entirely (for poly(G)) or at least partially (for poly(G).poly(C) and poly(G,C)) to the triplet emission of mutually interacting chromophores. The total quantum yield of poly(G) is slightly reduced relative to that of GMP, however no further decrease accompanies the formation of the hydrogen-bonded complex poly(G).poly(C). The quantum yield of the copolymer poly(G,C) is substantially lower.

The results obtained indicate that the energy of single stranded polynucleotide or oligonucleotide excimer states can be modified on formation of a hydrogen-bonded complex having ordered conformation with another polynucleotide. The observation of emission from poly(G).poly(C) implies that in the interpretation of DNA low temperature luminescence spectra the contribution of guanine-cytosine pairs should be considered. The quantum yield of DNA excimer emission depends primarily on interactions of neighbouring bases in one strand and is determined by base sequence.

The low temperature emission spectra of poly(G) were first described by Rahn, Longworth, Eisinger and Shulman[1], who used the mixture ethylene glycol–water as the solvent. As compared with the spectrum of GMP, both singlet and triplet emissions were red shifted, but their quantum yields remained unchanged. In the phosphorescence decay two components were detected. Experiments carried out later in the same laboratory on poly(G) and poly(G).poly(C) showed that fluorescence and phosphorescence intensity of poly(G) was reduced relative to GMP and that the formation of hydrogen bonds between guanine and cytosine residues in the complex poly(G).poly(C) resulted in complete quenching of the emission[2,3]. More recently the emission of poly(G)

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and poly(G).poly(C) was studied at somewhat different conditions, *i.e.* at pH 2·6 and room temperature[4]. Even though the ordered helical structure of poly(G) as well as the double helical hydrogen-bonded conformation of poly(G).poly(C) exist[5] up to pH 1, the quenching of emission reported for poly(G).poly(C) at pH 7 and liquid nitrogen temperature was not observed under these conditions.

The decrease of low temperature luminescence quantum yield was also observed for the dinucleotides containing guanine and cytosine, *i.e.* GpC and CpG, relatively to the luminescence of the equimolar mixture of GMP and CMP[6]. In contrast to the hydrogen-bonded polynucleotide complexes, in the dinucleotides only the interactions between stacked chromophoric residues are responsible for the observed spectral changes[6,7].

In the present paper the low temperature spectra of poly(G), poly(G).poly(C) as well as of the copolymer poly(G,C) at pH 7 are examined in order to elucidate the relative effectiveness of different pathways of the quenching of emission from guanine and cytosine residues in DNA. Since the aggregation of chromophores induced by freezing the solutions can influence significantly the emission spectra[8–10], various solvents forming rigid matrices upon cooling have been used to discriminate and eliminate the aggregation effect.

EXPERIMENTAL

The samples of cytidine 5′-monophosphate (CMP) (Schwarz Bioresearch, Orangeburg, N.Y.), guanosine 5′-monophosphate (GMP), poly(G), poly(C) and poly(G,C) (all from Miles Laboratories, Elkhart, Ind.) were used without further purification. The complex poly(G).poly(C) was obtained by mixing the equimolar solutions of poly(G) and poly(C) in 0·1M sodium acetate (pH 7). The formation of the 1 : 1 complex was checked by construction of mixing curves from absorption spectra at 280, 262, and 245 nm under the same conditions[11]. The other chemicals used were of analytical grade. The solvents were checked for low temperature emission. For recording the emission spectra at the temperature of liquid nitrogen Aminco-Keirs spectrofluorimeter was used. The details of the method were described in the preceding communication[12]. The samples were excited at the maximum of lowest energy absorption peak, or, for the determination of quantum yields, at 260 nm (38460 cm^{-1}). The recorded spectra were corrected for the sensitivity of the detecting system and the areas under respective peaks drawn in energy scale were used for the determination of quantum yields. The quantum yields were determined relatively to the fluorescence quantum yield of GMP in ethylene glycol–H_2O (1 : 1, v : v) glass, for which the value 0·13 has been reported[13]. From the reproducibility of the measurements the relative error of quantum yields was estimated as $\pm 25\%$. Phosphorescence lifetimes were determined from the plot of the logarithm of phosphorescence intensity *vs* time. In the experiments following solvent systems were used: *a*) pure water; *b*) mixture water–ethylene glycol (1 : 1, v : v) or 0·05M sodium acetate–ethylene glycol (1 : 1, v : v); *c*) 0·05M sodium acetate containing 0·25% glucose adjusted to pH 7 with acetic acid. Freshly bidistilled water with pH $\geqq$ 6·5 was always used. The solute concentration was $2 \cdot 10^{-4}$—$4 \cdot 10^{-4}$M in all media.

RESULTS

A. *Poly(G)*. The only difference between absorption spectra of poly(G) and GMP at pH 7 is a hypochromic effect observed in the near ultraviolet region for the poly-

mer; both poly(G) and GMP have the maximum[4] at 252 nm (39 680 cm^{-1}) and a shoulder at about 280 nm (35 710 cm^{-1}). However, as can be seen from the data in Table I and from the comparison of the emission spectra of GMP and poly(G) at 77 K (Fig. 1 and 2), the emission properties of guanine residues are modified by polymerization quite substantially. In pure water matrix, where the molecules of GMP are aggregated to considerable extent[8,9] and interactions between guanine chromophores comparable to those in poly(G) can be expected, similar features can be found in the spectra of GMP and poly(G) (Table I, Figs 1, 2). As compared with the spectrum of monomeric GMP obtained from H_2O–ethylene glycol matrix, both fluorescence and phosphorescence spectra of poly(G) are red-shifted and their quantum yields are decreased; the phosphorescence quantum yield is reduced by one order of magnitude. While the red shift of fluorescence is 1 100 – 1 400 cm^{-1} in corresponding matrices, the phosphorescence peak is shifted only slightly. It is difficult to evaluate exactly the magnitude of the shift, because at the same time the intensity distribution between the vibrational peaks is changed. Both substances also exhibit a non-exponential decay of phosphorescence, which can be resolved in two components: The long-lived one corresponds to the phosphorescence lifetime of monomeric GMP, while the short-lived one is characteristic for the triplet emission from the mutually interacting guanine moieties[9]. The proportion of the short-lived component is higher for poly(G). The addition of substances which decrease the solute aggregation (*i.e.* of polyalcohols, salts, and/or glucose)[8,9] disturbs the aggregation of GMP and, consequently, changes its emission spectrum; on the other hand, the basic characteristics of the spectrum of poly(G) remain nearly unchanged. This indicates that the observed spectral changes in poly(G) should be attributed to the mutual

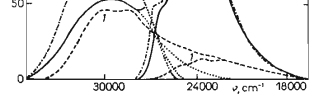

FIG. 1

Total Emission Spectra and Phosphorescence Spectra of $2 . 10^{-4}$M GMP in Pure Water *1* (×2) and 0·05M Sodium Acetate–0·25% Glucose Mixture *2* at 77 K. Curve *3* is the Spectrum in 0·05M Sodium Acetate and Ethylene Glycol Mixture (1 : 1, v : v)

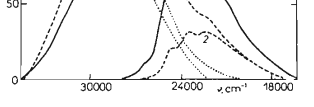

FIG. 2

Total Emission Spectra and Phosphorescence Spectra of $2 . 10^{-4}$M Poly(G) in Pure Water (*2*) (×5) and 0·05M Sodium Acetate–0·25% Glucose Mixture (*1*) (×3) at 77 K

TABLE I

Energies of Fluorescence and Phosphorescence Spectra, their Quantum Yields (φ_F and φ_P), Phosphorescence Lifetimes (τ) and the Phosphorescence-to-Fluorescence Ratio (P/F) for GMP, Poly(G), Poly(G).Poly(C), Poly(G,C), and the Mixture Poly(G) + CMP in Different Media at 77°K

Sample	Medium	Fluorescence[a]		Phosphorescence[a]							P/F
		ν, cm^{-1}	φ_F	ν, cm^{-1}			φ_P	τ_1, s [e]		τ_2, s	
GMP	*a*	*29 670*[f]	0·04	(25 190)	*23 810*[f]	22 830	0·009	0·5	(45%)	1·4	0·22
GMP	*b*	*30 300*[f]	0·13	(26 450)	*25 130*[f]	23 920	(22 880)	0·14	—	1·4	1·10
GMP	*c*	*29 850*[f]	0·09	(26 320)	*24 940*[f]	23 870	(22 600)	0·13	—	1·4	1·40
poly(G)	*a*	*28 570*[f]	0·04		24 690	*23 420*[f]	22 370	0·009	0·4	(75%)	1·4	0·22
poly(G)	*b*	*28 900*[f]	0·14	(26 110)	24 690	*23 420*[f]	(22 320)	0·08	0·5	(45%)	1·5	0·57
poly(G)	*c*	*28 570*[f]	0·05	(26 110)	24 690	*23 470*[f]	22 420	0·04	0·4	(65%)	1·4	0·80
poly(G).poly(C)	*a*	*26 670*[f]	0·04		(25 000)	(23 640)	*22 420*[f]	0·01	0·4	(55%)	1·1	0·25
poly(G).poly(C)	*b*	*26 950*[f]	0·10		(25 130)	(23 640)	*22 830*[f]	0·04	0·8[g]		—	0·40
poly(G).poly(C)	*c*	*27 620*[f]	0·06		25 000	*23 530*[f]	22 320	0·03	0·5	(50%)	1·3	0·50
poly(G,C)	*b*	(30 120)	*28 090*[f]	0·023	(26 110)	(24 510)	*23 530*[f]	(22 320)	0·031	0·5	(20%)	1·5	1·3
poly(G,C)	*c*	(29 940)	*27 930*[f]	0·030	(26 110)	(24 510)	*23 530*[f]	(22 320)	0·033	0·5	(15%)	1·4	1·1
poly(G) + CMP (1 : 1)	*a*		*27 780*[f]	—		(24 390)	(23 360)	*22 320*[f]	—	0·5[g]		—	—
poly(G) + CMP (1 : 1)	*c*		*30 770*[f]	—		24 940	*23 700*[f]	22 470	—	0·5	(40%)	1·4	—
poly(C) (ref.6)	*c*		*28 170*[f]	—			*23 810*[f]		—	0·6		—	very low

[a] Pure water. [b] 0·05M sodium acetate–ethylene glycol (1 : 1, v : v). [c] 0·05M sodium acetate with 0·25% glucose. [d] The values in parentheses denote shoulders in the fluorescence and phosphorescence spectra. [e] The relative proportion of the short-lived component is given in parentheses. [f] Wavenumber of the maximum. [g] Components of the complex decay could not be separated.

interactions of guanine residues stacked above each other in the ordered poly(G) conformation. The magnitude of poly(G) quantum yields as well as the percentage of the short-lived component in the phosphorescence decay depend somewhat on the matrix composition. From the study of the aggregation of purine derivatives in different frozen matrices[9] it can be concluded that the differences between a mononucleotide and the corresponding polymer are best expressed in an aqueous matrix containing only salts and/or glucose. In the case of poly(G) in such a type of matrix the proportion of the short-lived component is higher and also the fluorescence and phosphorescence quantum yields are lower than in the matrix containing polyalcohol.

B. *Poly(G).poly(C).* In the near ultraviolet region of the absorption spectrum of poly(G).poly(C) only one maximum at 259 nm (38 610 cm^{-1}) was observed[4]. The low temperature emission spectra of poly(G).poly(C) at neutral pH obtained from different matrices (Table I and Fig. 3) exhibit prominent fluorescence, the maximum of which is shifted to lower energies as compared with the maxima of both poly(G) and poly(C)[12]. In the sodium acetate–glucose matrix these shifts are 950 cm^{-1} and 550 cm^{-1}, respectively. In corresponding matrices the quantum yields have approximately the same value as for poly(G) and consequently the P/F ratios remain also low. The phosphorescence decay always contains a high percentage of the short-lived component, to which also triplet emission from the non-interacting cytosine residues can contribute, however. In the ethylene glycol–H_2O matrix it was difficult to detect and evaluate accurately the long-lived component. The failure to substract the long-lived component in the decay plot resulted in the averaged value of the observed lifetime (Table I). For comparison, the emission spectra of equimolar mixture of poly(G) with CMP were recorded (Table I). With exception of the measurement made in the pure water matrix, no significant chromophore interactions should be expected in this mixture. Consequently, the observed spectra correspond

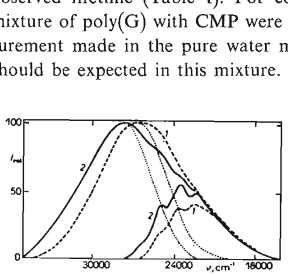

FIG. 3

Total Emission Spectra and Phosphorescence Spectra of 2 . 10^{-4}M Poly(G).poly(C) in pure water (*1*) (×5) and 0·05M sodium acetate–0·25% glucose mixture (2) (×3) at 77 K.

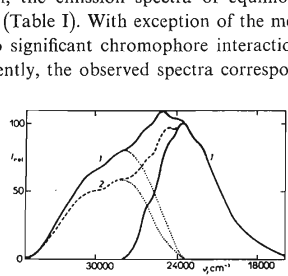

FIG. 4

Total Emission Spectra and Phosphorescence Spectra of 2 . 10^{-4}M poly(G,C) in 0·05M sodium acetate containing 0·25% glucose *1* (×5) or 50% ethylene glycol 2 (×5). The phosphorescence is identical for both matrices.

very closely to the sum of the spectra of the components[12,13]. The luminescence spectrum of the mixture obtained from the pure water matrix indicates that some interactions between the two types of bases exist in the medium favoring solute aggregation.

C. *Poly(G,C)*. The absorption spectrum of poly(G,C) is similar to that of poly(G).poly(C) having single peak at 258 nm (38 760 cm^{-1}). The low temperature luminescence spectrum of poly(G,C) (Table I, Fig. 4) exhibits features characteristic for the luminescence of the corresponding dinucleotides CpG or GpC (ref.[14]). The singlet emission consists of two well resolved peaks. The lower energy peak corresponds to the emission from the excimer state of the interacting guanine and cytosine moieties, the other one represents the emission of the non-interacting residues. The energy of the excimer peak of poly(G,C) is higher than that of poly(G).poly(C) in the corresponding media. On the other hand, it is lower than the energy of excimer peak of poly(G) even though the position of the apparent maximum of the excimer peak of poly(G,C) can be influenced by an overlap with the "monomer" peak. The phosphorescence spectrum of poly(G,C) exhibits the vibrational structure characteristic for guanine, but the whole band is slightly red-shifted and the distribution of intensity between the vibrational peaks is modified in the same way as for poly(G). The quantum yields of both fluorescence and phosphorescence of poly(G,C) are significantly lower than those of poly(G).poly(C). This decrease is more pronounced in the polyalcohol glasses. From the comparison of the P/F ratios it follows that the spectrum of poly(G,C) is much more similar to that of CpG ($P/F = 1{\cdot}05$) than of CpG ($P/F = 2{\cdot}5$)[6,14].

DISCUSSION

In the previous experiments it was observed that the chromophore interactions in polynucleotides as well as in the samples of the constituting monomers can be influenced by the solvent which formed the low temperature matrix[8,9]. Therefore the present measurements were carried out in several types of matrices in order to recognize and eliminate the effects caused by the formation of solid matrix. Unlike to poly(A)[12], only minor differences were observed between the spectra of the guanine containing polynucleotides obtained from the aqueous salt and/or glucose containing matrices and from the polyalcohol–water matrices. However, these differences (*e.g.* the magnitude of the red shift of fluorescence maximum or the proportion of the short-lived component in phosphorescence decay[9]) indicate that in the former type of matrix the ordered structure of poly(G) is better preserved.

The spectra obtained from the pure water matrix assume an exceptional position, because upon cooling the preferential crystallization of pure water takes place and in the remaining liquid regions of the sample the concentration of solute molecules increases, which results in "forced" aggregation of the solute[8,9]. Solute molecules are most

probably stacked in the aggregates so that the emission spectra of the aggregated monomeric constituents are usually similar to those of the corresponding polynucleotides. By comparing the spectra of the polynucleotides in the pure water matrix and in the matrices disturbing efficiently solute aggregation with the spectra of aggregated corresponding residues, the extent of chromophore interactions in the polymer in different environments can be evaluated. Thus one can distinguish between spectral changes due to aggregation induced by the medium and those due to the interactions of monomeric residues held together by covalent bonds and assuming more or less ordered conformation at room temperature.

As it has been found earlier[7-9], spectral changes caused by the mutual interaction of chromophoric residues in polynucleotides or in the aggregates of the corresponding monomers, can be characterized by: *a*) red shift of fluorescence maximum, which can be attributed to exciton er excimer[15] formation; *b*) relatively smaller red shift of phosphorescence maximum; and *c*) non-exponential decay of triplet emission. These changes can be accompanied by variations in fluorescence and phosphorescence quantum yields; usually the P/F ratio is much lower for the interacting chromophores than for the corresponding monomers[9].

As compared with GMP in monomeric state (*i.e.* in the polyalcohol glass), the emission spectra of poly(G) and poly(G).poly(C) exhibit appreciable red shift of fluorescence independently on the matrix composition. The peak is broad, structureless and nearly symmetric. No comparable shift is observed in the absorption spectrum of poly(G)[4], which indicates only little interaction in the ground state. Also the relation between the near ultraviolet absorption peak of poly(G).poly(C), which lies between the peaks seen in the mixture of GMP and CMP, and its fluorescence spectrum does not give evidence on exciton formation. These factors indicate that the singlet emission takes place from an excimer state[6,7,16].

In the fluorescence spectrum of poly(G,C) the excimer emission at about 28000 cm^{-1} appears beside the peak which corresponds to the emission from non-interacting GMP and CMP residues. The excimer peak lies at somewhat higher energies than that of poly(G).poly(C), but it remains red-shifted relative to the fluorescence maximum of poly(G). Similar red shifts in fluorescence spectrum interpreted as due to excimer formation were observed for dinucleotides[6,7,14]. Guanine containing dinucleotides always yield both the excimer peak and the peak corresponding to the monomer mixture emission[14]. The magnitude of the fluorescence red shift is comparable with the shifts of the excimer peaks in GpA and ApG (ref.[6,14]) for poly(G), and of GpC and CpG (ref.[14]) for poly(G).poly(C) and poly(G,C). It should be noted that the spectrum of poly(G,C) has positions of the maxima and the P/F ratio (which is different for CpG and GpC (ref.[6,14])) identical with CpG. On the other hand, poly(G) and poly(G).poly(C) do not exhibit detectable remainders of monomer emission. There can be several reasons for the observed small variations in the position of the excimer peak in spectra of the polynucleotides measured in different matrices: The maximum can be shifted to higher energies due to *a*) an overlap with the fluorescence peak corresponding to the emission from the non-interacting chromophores,

b) change of the energy of molecular exciton and charge resonance states (which both contribute to the excited dimer states[17]) with changes in the separation of interacting molecules in different types of matrices; *c*) additional effects, as *e.g.* solvent effect, which can be of different magnitude for the regular arrangement of chromophores and for randomly oriented molecules.

The small red shift of phosphorescence of poly(G).poly(C), poly(G), and poly(G,C) as compared with the GMP phosphorescence can be accounted for by a solvent effect as mentioned under *c*) above. A change in the equilibrium configuration of the lowest excited triplet due to solvent effects can also explain the different distribution of intensity between the vibrational bands in GMP on the one hand and the polynucleotides on the other hand.

The phosphorescence spectra of poly(G).poly(C) and poly(G,C) have all characteristics of the phosphorescence of poly(G). Most likely this finding can be explained by an energy transfer on the triplet level. This type of energy transfer was demonstrated in polynucleotides, oligonucleotides as well as nucleoside aggregates[6,7,16], in which triplet emission is characteristic for the residues having lowest energy triplet levels. The order of energies of nucleotide triplets[16] ($U^- > C > G > T^- > A > T$) indicates that only emission from guanine residues should be expected. However, because of relatively much lower quantum yield of phosphorescence of CMP or poly(C)[12,13,18,19], the eventual contribution from cytosine residues could be under limits of detection. This probably explains, why also the phosphorescence of the mixture of poly(G) with CMP has the same character as that of the polynucleotide complex.

As it has been shown earlier[9] the short-lived component present in the phosphorescence decays of GMP in pure water matrix and of poly(G) corresponds apparently to the emission from the mutually interacting guanine residues. Its presence can be correlated with the appearance of the excimer peak and, as it has been shown in the experiments with aggregates of purine derivatives in different matrices[9,10], its proportion can be related to the fraction of interacting residues in the sample. The short-lived component of poly(G) lifetime is very close to that of cytosine derivatives[13,19], and therefore it is impossible to evaluate relative contributions to the complex phosphorescence decay in the polynucleotides containing cytosine residues. However, the higher proportions of the short-lived component in the pure water matrices give evidence that a mechanism similar to that considered for poly(*G*) is at least partially responsible for its origin.

The quantum yields of both fluorescence and phosphorescence of poly(G) are decreased with respect to that of non-aggregated GMP, if the aqueous matrix with salt and glucose is used; in the polyalcohol containing solvent only the phosphorescence quantum yield is reduced. In pure water matrix the quantum yields of poly(G) further decrease, especially that of phosphorescence, and approach to the values found for the aggregated GMP. The formation of the hydrogen bonded complex poly(G).poly(C) does not lead to any significant decrease of quantum yields; the other spectral changes indicate that an interaction between guanine and cytosine residues in excited state takes place, however. Only in the copolymer poly(G,C) the quantum yields are reduced

to values which are lower than those of the corresponding dinucleotides CpG and GpC (ref.[14]).

It is difficult to correlate the observed changes in low temperature luminescence with the conformation of the polynucleotides, even if it is supposed that in suitable medium the polynucleotide conformation is not changed essentially upon freezing the samples. At room temperature and neutral pH poly(G) is apparently present in solution in highly ordered multistranded conformation[5,11]. Poly(G).poly(C) forms under the same conditions a double stranded helix with guanine and cytosine residues joined by hydrogen bonds[5,11], while poly(G,C) with random distribution of the two bases can form hydrogen bonded guanine–cytosine pairs only in small regions, possibly also incorporating loops, which are separated by single strand regions; it is not known whether the bases in single strand are stacked.* In the both polynucleotides with the regular helical multistranded structures the excimer emission predominates, while poly(G,C) fluorescence contains also the peak corresponding to the monomer emission. On the other hand, strong excimer interaction resulting in the absence of any monomer emission was observed for some dinucleotides in polyalcohol glasses (*i.e.* ApU, ApC, and CpU (ref.[6,7,14])), where only interactions of stacked residues could be considered. Thus, from the results obtained, it only can be concluded that the stacking of a pair of bases is sufficient for the formation of an excimer and that the energy of the excimer state can further be modified if the bases participate by means of hydrogen bonds in formation of highly ordered helical structure (*i.e.* in the case of poly(G).poly(C), the excimer peak of which is red-shifted relative to excimer peaks of the constituting homopolynucleotides). In the less ordered structure of poly(G,C) the energy of the excimer state remains the same as for the dinucleotides CpG and GpC.

The existence of certain interactions of excited singlet states in hydrogen bonded base pairs can explain the observed quenching of luminescence of poly(A) or poly(C) in the complexes with poly(U) or poly(I)[1,12,20,21]. The latter two polynucleotides can act as efficient quenchers because their rates of nonradiative energy dissipation are very high: no low temperature luminescence has been detected from them[12] or even from UpU (ref.[6,14]). In contrast to the behaviour of these complexes, no quenching of adenine residues was found in single-stranded copolymers, which contained randomly distributed uracil residues[21].

The observation of the low temperature luminescence from poly(G).poly(C) at neutral pH requires a modification of interpretation of the spectra of DNA. Since the formation of hydrogen bonded guanine–cytosine pair does not quench completely the luminescence of both residues, a contribution of all constituent bases to the fluorescence peak of DNA (ref.[12]) at 28200 cm^{-1} which is of excimer nature should

* Upon heating in 10^{-2}M cacodylate buffer (pH 6·9) the sample of poly(G,C) yielded a broad melting profile with hyperchromicity of 17% at 38600 cm^{-1} (259 nm) and at the temperature 99·5°C.

be considered. The results obtained indicate that the explanation of the reported qualitative differences in luminescence quantum yields of DNAs with different base composition[16,22] should be sought rather in the influence of various base sequences on excimer emission quantum yields than in complete quenching of emission from guanine–cytosine pairs. The data on fluorescence quantum yields for various diribonucleoside phosphates[6,7,14] give evidence that already the interaction of two bases can affect the singlet emission quantum yields as compared with the corresponding mixture of nucleotides. The fluorescence quantum yields differ within limits of one order of magnitude for various combinations of nucleosides and are further modified upon formation of trinucleotides[14]. The possibility of an influence of base sequence on the energy migration on the DNA triplet level was also suggested by Isenberg, Rosenbluth, and Baird[22] on the basis of measurement of phosphorescence quenching in DNAs of different base composition by paramagnetic cations. At present no information is available on the number of excimer states in DNA or their energies[7]. The possibility of an analysis is, besides other factors, complicated by the fact that the energies of excimer states of various nucleotide combinations in dinucleotides are very close (within $\pm 400\ cm^{-1}$) to the energy of the excimer peak of DNA (ref.[14]).

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