LUMINESCENCE PROPERTIES OF DINUCLEOSIDE PHOSPHATES, SOME OLIGONUCLEOTIDES AND POLYNUCLEOTIDES*

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Low temperature emission spectra of 16 diribonucleoside phosphates were measured both in propylene glycol–phosphate buffer (pH 7) mixture (1 : 1, v/v) and in buffered aqueous media containing 0·25% glucose. The measurements were extended to trinucleoside diphosphates containing adenine and uracil and to copolymers poly(A,G), poly(A,C), poly(A,U), poly(C,U), poly(A,G,C,U), and poly[d(A–T). d(A–T)] in order to establish the effect of base composition on the emission properties of longer polymers. The excimer emission was observed for all dinucleoside phosphates (except UpU). The properties of the oligomers and the copolymers were in most cases similar to those found for dinucleoside phosphates; only poly(G,C) exhibited lower quantum yields. It is assumed that the base sequence might be an important factor in determining the properties of the excited states of nucleic acids.

In our preceding communication[1] it has been shown that the low temperature emission from the hydrogen bonded pair guanine–cytosine is not completely quenched and that interactions of all constituent bases should be considered in the interpretation of the emission spectra of DNA. In this respect the understanding of the luminescence properties of known short sequences of nucleotides as well as the studies of polynucleotides composed of limited number of purine or pyrimidine bases seem to be essential for the elucidation of emission spectra of nucleic acids.

In the earlier experiments[2–5] the basic features of the low temperature luminescence of dinucleoside phosphates were established. Whereas only small changes can be observed in the absorption spectra, *i.e.* hypochromicity of several per cent and eventually a slight shift of the near ultraviolet maxima[6,7], it has been shown that the singlet emission of several dinucleoside phosphates contains a broad, structureless band, substantially red-shifted relative to fluorescence maximum of the corresponding mononucleotides, which is attributed to excimer** emission[2].

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** In the present communication the term "excimer" will be used generally in accordance with the previous publications, even though it should be differentiated between complexes formed between two nonidentical chromophores ("exciplexes") and those formed between two identical chromophores ("excimers").

The triplet emission of dinucleoside phosphates exhibits all characteristics corresponding to emission from only one constituent monomer. Studies on various base combinations have shown that the predominating emission always occurs from the residue with lower-lying triplet level, in agreement with the order of relative lowest-triplet energies of purine and pyrimidine bases, $U^- > C > G > A > T$ (ref.[5]).

Differences in the relative predominance and energy of excimer emission have been observed for various dinucleoside phosphates, even for sequence isomers[4,5]. Moreover, it has been demonstrated that the composition of the low temperature solvent matrix can affect the excimer emission of dinucleoside phosphates[4]. These observations indicate that the changes can be caused by variations either in the extent or in the strength of chromophore interactions.

We present here data on luminescence of 16 dinucleoside phosphates which exhibit some features not described in the previous communications: The excimer peak was studied in all examined dinucleoside phosphates and the relative contributions of excimers on the one hand and the noninteracting residues on the other hand to the singlet emission were compared especially for the sequence isomers. In order to obtain information on the effect of polymer length on excimer emission, the measurements have been extended to some trinucleoside phosphates and the corresponding nucleotide copolymers.

EXPERIMENTAL

Chemicals and methods: All dinucleoside monophosphates (except GpG) and ApdUpA were obtained from Calbiochem, Los Angeles, California. GpG, trinucleoside diphosphates ApApU, UpApA, and ApUpU and copolymers poly(A,G), poly(A,C), poly(A,U), poly(G,C), poly(C,U), poly(A,G,C,U), and poly[d(A – T). d(A – T)] were the products of Miles Laboratories, Elkhart, Indiana. The low temperature luminescence measurements were carried out in the mixture propylene glycol–0·1M phosphate buffer (pH 7) (1 : 1, v/v) or in the solution of 0·05M sodium acetate containing 0·25% glucose. For measurements at low pH 0·05M acetate buffer was used to adjust proper pH value. Absorption spectra at room temperature were measured on Cary 15 spectrophotometer. Aminco-Keirs spectrofluorimeter with high resolution setting was used for the emission measurements at liquid nitrogen temperature. The details of the experimental procedure were described earlier[1]. The emission spectra were corrected for spectral sensitivity of the detecting system. Quantum yields were estimated relative to that of fluorescence of GMP in ethylene glycol–water (1 : 1, v/v) glass for which the value 0·13 was reported[8]. In all cases the samples were excited at 38460 cm^{-1} (260 nm).

RESULTS AND DISCUSSION

Low Temperature Luminescence Spectra of Dinucleoside Phosphates

The data on emission spectra of sixteen 3′-5′ diribonucleoside phosphates obtained from the matrix propylene glycol–phosphate buffer at liquid nitrogen temperature are given in Tables I and II together with the spectra of mixtures of corresponding nucleotides. The spectra of dinucleoside phosphates are illustrated on Figs 1 and 2. With only exceptions of ApA (in which the red shift of fluorescence can be attributed

to exciton formation[4]) and UpU (which gives no detectable emission), all studied dinucleoside phosphates yield excimer emission, either as the predominating component of fluorescence, eventually as the only one, or as a shoulder lying on the low energy side of the peak, which apparently corresponds to emission from the non-interacting constituent chromophores. Similar results were obtained from the aque-

TABLE I

Luminescence Data of Diribonucleoside Phosphates and of Corresponding Mixtures of Nucleotides Obtained from Propylene Glycol–Phosphate Buffer (pH 7) (1 : 1, v / v) Matrix at 77 K

Compound	Fluorescence[a]			Phosphorescence		
	monomer maximum ν cm^{-1}	excimer maximum ν cm^{-1}	quantum yield, φ_F	type of emission	lifetime[b] τ, s	quantum yield φ_P
AMP	*31 750*	—	0·012	A	2·5	0·014
ApA	—	*30 400*[c]	0·07	A	2·6	0·13
GMP	*30 300*	—	0·13	G	1·3	0·16
GpG	*30 300*	(28 570)	0·07	G	1·4	0·11
CMP	*31 060*	—	0·06	C	0·6	0·02
CpC	—	*28 090*	0·12	C	0·7	0·03
UMP	*31 150*	—	0·01	U	—	<0·01
AMP+GMP	*29 760*	—	0·10	A+G	1·2(65%) 2·7	0·12
ApG	*29 410*	(28 090)	0·04	A+G	1·1(10%) 2·7	0·08
GpA	*29 410*	(27 930)	0·04	A+G	1·1(10%) 2·6	0·11
AMP+CMP	*31 450*	—	0·05	A+C	0·6(20%) 2·7	0·03
ApC	—	*27 900*	0·07	A	2·6	0·17
CpA	29 850	*27 800*	0·03	A	2·6	0·07
AMP+UMP	*33 300*	—	0·02	A	2·6	0·015
ApU	—	*27 860*	0·03	A	2·6	0·04
UpA	29 940	*27 900*	0·03	A	2·5	0·04
GMP+CMP	*30 120*	—	0·13	G	1·3	0·13
GpC	*29 670*	27 930	0·05	G	1·3	0·13
CpG	*29 590*	27 930	0·09	G	1·3	0·12
GMP+UMP	*29 760*	—	0·10	G	1·3	0·09
GpU	*29 760*	28 330	0·07	G	1·2	0·08
UpG	*29 760*	(28 330)	0·03	G	1·2	0·025
CMP+UMP	*31 060*	—	0·03	C	0·6	0·015
CpU	—	*28 400*	0·04	C	0·7	0·02
UpC	*30 300*	(28 010)	0·02	C	0·7	0·01

[a] Values corresponding to maxima of higher intensity are italicized; values corresponding to shoulders are given in parentheses. [b] If two components were resolved in the phosphorescence decay, their relative proportions are indicated in parentheses. [c] The fluorescence of ApA is probably from exciton state.

TABLE II

Ratio of Exciplex Emission Quantum Yield to Total Fluorescence Quantum Yield (φ_{Ex}/φ_F) for Dinucleoside Phosphates in Different Matrices at 77 K

Compound	φ_{Ex}/φ_F		Compound	φ_{Ex}/φ_F	
	a	*b*		*a*	*b*
ApA	—	0·9[c]	ApU	0·8	0·9
GpG	0·5	0·8	UpA	0·3	0·5
CpC	1·0	1·0	GpC	0·5	0·5
UpU	—	—	CpG	0·5	0·5
ApG	0·4	0·8	GpU	0·4	0·5
GpA	0·3	0·8	UpG	0·4	0·5
ApC	0·8	0·9	CpU	0·9	0·4
CpA	0·6	0·7	UpC	0·5	1·0

[a] Propylene glycol–0·1M phosphate buffer (pH 7) (1 : 1, v / v); [b] 0·1M phosphate buffer (pH 7), 0·05M sodium acetate, 0·25% glucose; [c] medium without glucose.

ous matrices not containing propylene glycol, *i.e.* frozen buffered solutions which contained higher salt concentrations or 0·25% glucose. The change of matrix composition induces only changes in relative contribution of excimer emission to the fluorescence of dinucleoside phosphates (Table II). In the aqueous matrices the relative proportion of the excimer emission usually increases: In most cases (with exception of GpU and UpC) the excimer peak became dominant in the singlet emission.

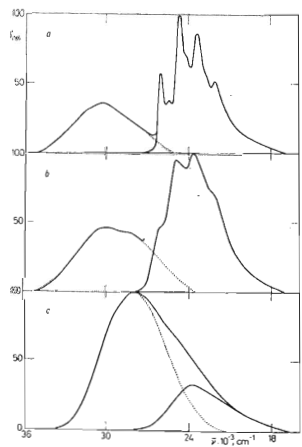

FIG. 1

The Fluorescence and Phosphorescence Spectra of ApA (*a*), GpG (*b*), and CpC (*c*) in Propylene Glycol–0·1M Phosphate Buffer (pH 7) Matrix at 77 K

The excitation wavelength was 260 nm (38 460 cm^{-1}). The spectra were normalized and the intensity is given in arbitrary units (I_{rel}).

The energy of the excimer peak is not affected by matrix composition, however. Small variations in energy of excimer maximum (within 500 cm^{-1}) were found for different dinucleoside phosphates; for all nucleoside combinations the values of energy are very close to that found[9] for the excimer peak of DNA, *i.e.* 28200 cm^{-1}.

Also the character of phosphorescence is independent on matrix composition. In most cases the phosphorescence corresponds to the emission from the residues with lowest-lying triplet state. The mechanism of the population of the lowest triplet level is not yet elucidated; however, two possibilities were suggested[5]: *a*) preferential intersystem crossing from the excimer state to the lowest triplet state, or *b*) energy transfer on triplet level between the interacting components of the dimer. The resi-

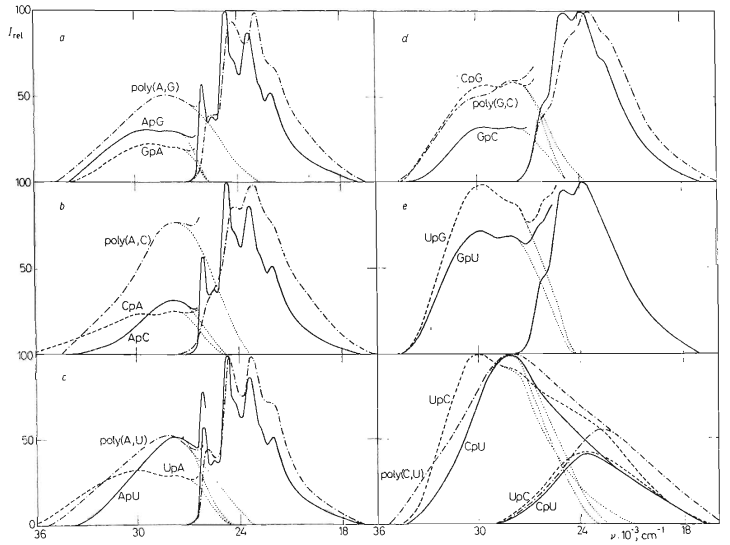

FIG. 2

Comparison of Low Temperature Luminescence Spectra of Dinucleoside Phosphates and the Corresponding Nucleotide Copolymers

The conditions are the same as given in the legend to Fig. 1. (*a*) ——— ApG; — — — GpA; —.— poly(A,G); (*b*) ——— ApC; — — — CpA; —.— poly(A,C); (*c*) ——— ApU; — — — UpA; —.— poly(A,U); (*d*) ——— GpC; — — — CpG; —.— poly(G,C); (*e*) ——— GpU; — — — UpG; (*f*) ——— CpU; — — — UpC; —.— poly(C,U).

dues of dinucleoside phosphates, which for any reason do not interact with each other, should phosphoresce independently. By analyzing the phosphorescence decays we were able to detect small contribution of guanine moieties to triplet emissions from GpA and ApG. It is difficult, however, to detect eventual small contributions to phosphorescence from pyrimidine derivatives, which have appreciably lower phosphorescence quantum yields than purine bases[8].

The high energy component of fluorescence peak is usually located close to the position of fluorescence maximum of the mixture of corresponding nucleotides. It corresponds apparently to emission from the constituting chromophores of dinucleoside phosphates which do not mutually interact. The positions of these two maxima are identical, if the excimer emission quantum yield is relatively low. In the other case the monomer peak is usually slightly red-shifted, probably due to an overlap with the broad excimer peak. If the resolution of emission spectra is not sufficient, the overlap can eventually lead to merging of the monomer and excimer peaks, which results in rather moveable position of fluorescence maximum, *e.g.* as a function of external conditions. This might serve as explanation of some difference between results reported earlier[4,5].

ApA, which has stacked conformation at room temperature as can be guessed from circular dichroism data[6,7], exhibits only small red shift of fluorescence band relative to AMP in the propylene glycol–water matrix (Table I). The position of the fluorescence maximum of ApA is identical with that of single stranded poly(A) in the same type of matrix[9,10] and the magnitude of the shift is compatible with the width of excition splitting for AMP polymers[8,11]. In the aqueous matrices containing salt and 0·25% glucose the excimer emission becomes apparent as a weak shoulder at 28 400 cm^{-1}; in the aqueous matrices without glucose the excimer peak predominates (Fig. 3). The protonization of ApA also enhances the excimer emission: at pH 3·5 the excimer peak at 27 900 cm^{-1} represents the only singlet emission from ApA in aqueous as well as in polyalcohol matrices. Similar shift of fluorescence maximum with decreasing pH was also observed[9,10] for poly(A).

The changes of fluorescence spectra of ApA under different environmental conditions suggest that various types of chromophore interactions contribute to excimer emission in different proportions depending on the external factors. It has been shown[12] that the excimer states can be derived from configuration interaction of molecular exciton states and charge resonance states. Formation of charge transfer complexes, which is more strongly expressed in excited states has been demonstrated[13] in cytidine aggregates and polymers at pH values corresponding to cytosine p*K*. It seems likely that the partial protonization of adenine residues in ApA (which takes place at pH $\approx$ 3·5) in propylene glycol glass increases the contribution of charge resonance states to the excimer states. On the other hand, the appearance of strong excimer emission in frozen aqueous salt-containing media at pH 7 should be attributed to stronger exciton-type interaction, which can be a result of a modification of sterical configuration of ApA in the different type of matrix supporting solute aggregation[14,15].

The other two homogeneous dinucleoside phosphates from which emission has been observed, *i.e.* CpC and GpG behave in a different way. CpC exhibits only the excimer emission whereas GpG yields both excimer and monomer peaks in the glycol matrix and single excimer peak in aqueous matrices. However, for CpC an increase of both fluorescence and phosphorescence intensity was observed[13] in propylene glycol glass at pH values in the vicinity of its p*K*; this, analogically to ApA might reflect the enhanced contribution of charge resonance states to the excimer ones.

The behaviour of dinucleoside phosphates clearly indicates that the conformation of the constituting chromophores, which conditions the formation of the excimers, depends on the solvent composition and, consequently, on the structure of the low temperature matrix. Similar dependence of fluorescence spectra on low temperature matrix composition was observed for monomeric derivatives of purine and pyrimidine[14,15]. The low temperature luminescence of dinucleoside phosphates can thus be considered as an overlap of emissions from molecules with chromophoric units assuming conformation that enables the formation of eximers and, on the other hand, from molecules in which the chromophoric units do not interact. The relation of emission properties of dinucleoside phosphates to another data giving information on their conformation will be discussed in the next paragraph. The quantum yields of dinucleoside phosphate fluorescence and phosphorescence are of the same order of magnitude as those of the corresponding mixtures of mononucleotides; their values can be higher or lower. For some dinucleoside phosphates (*e.g.* ApG, GpA, and ApU) we have obtained quantum yields close to the values reported earlier[5]; in other cases our results are different. The measurements made in the aqueous media showed that the quantum yields depended on the nature of salts present in the solvent. We did not study this effect in a greater detail; we have observed that in the matrix containing only phosphate and/or chloride anions the quantum yields were always significantly lower than in the matrix containing acetate anion. It is thus possible that the differences in solvent systems can account for the discrepancies in the quantum yields.

Comparison of the Low Temperature Luminescence with other Physical Properties of Dinucleoside Phosphates

In past several years great effort has been concentrated on the elucidation of structure and conformation of dinucleoside phosphates in solution. The basic techniques used were NMR[16,17], optical rotatory dispersion and circular dichroism[7,18], and studies of hypochromicity in ultraviolet absorption spectra [6,7,17]. The information on the conformation of the dimers was obtained through careful comparison of properties of monomers and dimers. Similar approach has been used in this and preceding studies[5,9] on emission properties of oligo- and polynucleotides. Thus it seems to be of interest to correlate the occurence of excimer emission with other data characterizing the mutual interaction of chromophores in the dinucleoside phosphates.

It has been shown by circular dichroism measurements[18,19] that it is the 3′-linked nucleoside which determines the geometry of a dinucleoside phosphate. Accordingly, the interaction of mo-

TABLE III

Luminescence Data of Trinucleoside Diphisphates in Propylene Glycol–Phosphate Buffer Matrix (pH 7) (1 : 1, v/v) at 77K

Compound	Fluorescence[a]			Phosphorescence[a]							
	$\bar{\nu}$, cm^{-1}		quantum yield, φ_F	$\bar{\nu}$, cm^{-1}						quantum yield, φ_P	lifetime τ, s
ApApA	*30 400*	—	—	26 180	25 510	*24 690*	(24 150)	23 310	22 120	—	2·8
ApApU	—	*27 780*	0·01	26 110	25 450	*24 750*	(24 210)	23 420	22 220	0·02	2·5
ApdUpA	—	*28 170*	0·01	25 710	—	*24 510*	—	23 250	22 000	0·03	2·5
UpApA	*30 400*	(28 100)	0·01	26 110	25 450	*24 750*	(24 210)	23 420	22 120	0·02	2·5
ApUpU	—	*27 930*	0·02	26 300	25 770	*24 940*	(24 390)	23 640	22 320	0·02	2·3

[a] See the footnote in Table I.

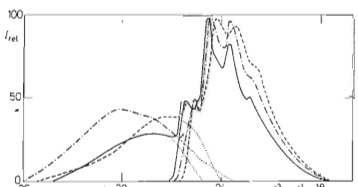

FIG. 3

Illustration of the Changes of Low Temperature Luminescence Spectra of ApA with pH and under Different Environmental Conditions

——— Propylene glycol–0·1M acetate buffer (pH 3·5) (1 : 1, v/v); — — — 0·1M phosphate buffer (pH 7), 0·3M-NaCl; —.— 0·05M phosphate buffer (pH 7), 0·05M sodium acetate, 0·25% glucose. The excitation wavelength was 260 nm (38460 cm^{-1}).

nomeric units should be different in sequence isomers. The extent of interaction, which is expressed by the amplitude of the spectral curve[7,18] as well as the proportion of excimer emission is always higher in ApC and ApU as compared with CpA and UpA. This result is also in agreement with the NMR measurements[16]. On the other hand, similar correlation could not be found for dinucleoside phosphates containing guanine. Thus, *e.g.* the great difference between the CD spectra of ApG and GpA is not reflected by the emission spectra, which are very similar. It is noteworthy to mention here that, in contrary to ApC, ApU, or CpU, in the dinucleoside phosphates containing guanine as one component the excimer emission never represented the only singlet emission: always the mixture of excimer and monomer emissions was observed.

The classification of dinucleoside phosphates using the two-state conformation model has been carried out by Warshaw and Tinoco[7] on the basis of hypochromicity and optical rotatory dispersion measurements. A simple thermodynamic equilibrium between the stacked and unstacked forms of dinucleoside phosphates has been assumed. It has been concluded that uracil containing dinucleoside phosphates have the least tendency to stacking. This model is consistent with the self-association studies[20] giving the following order of base–base interactions: purine–purine > purine–pyrimidine > pyrimidine–pyrimidine. Unfortunately this classification does not give any clue for the interpretation of emission properties of dinucleoside phosphates. The two-state model also failed to explain the different temperature dependencies of optical properties of sixteen diribonucleoside phosphates[21]. The alternative explanation proposed by Glaubiger, Lloyd and Tinoco[22] considers oscillation of the bases in some sort of potential field. The basic assumptions of this torsion oscillator model are justified by considerations of possible steric barriers to rotation along the glucosidyl bond[23] and by analysis of allowed conformations of monomeric units in polynucleotides[24], as well as by the NMR measurements[16].

The latter model can reasonably explain the emission properties of dinucleoside phosphates. It can be assumed that the varying proportions of excimer and monomer emission are determined by the fractions of dimer molecules fixed at low temperature in configurations involving greater (stacking) or lesser (unstacking) overlap of the chromophores. The dominating excimer emission from several dinucleoside phosphates (ApC, ApU, CpU, CpC) might then reflect the stronger interaction of constituting chromophores in the excited as well as in the ground state. It should be, however, pointed out here that the ratio of excimer and monomer emissions is not constant characteristics of given dinucleoside phosphate, but that it is strongly dependent on the composition of the low temperature matrix surrounding the chromophores (Fig. 3).

The weak point in the comparison of luminescence spectra with other physical properties of dinucleoside phosphates is the difference in environmental conditions used. Unfortunately, the low temperature rigid matrix is essential for luminescence measurements, since it is the simplest way how to increase the low quantum yields of the studied compounds. The attempt to compare directly the emission from monomeric purine and pyrimidine derivatives and several dinucleoside phosphates at room temperature with the hypochromicity and optical rotatory dispersion data of Warshaw and Tinoco[7] made by Glaubiger[25] failed due to very low emission quantum yields of the studied compounds. However, the recently published data on the room temperature emission from purine and pyrimidine bases and their derivatives[26–29]

TABLE IV

Luminescence Data of Several Nucleotide Copolymers in Different Matrices at 77K

Compound		Fluorescence[a]			Phosphorescence[a]					lifetimes[b]		φ_P/φ_F
		$\bar{\nu}$, cm^{-1}		quantum yield, φ_F	$\bar{\nu}$, cm^{-1}				quantum yield, φ_P	τ_1, s	τ_2, s	
Poly(A,G)	[c]		*28 330*	0·10	25 770	24 330	*23 040*	(21 900)	0·12	1·3 (30%)	2·7	1·2
	[d]		*28 170*	0·13	(25 600)	24 270	*23 150*	(22 000)	0·11	1·0 (35%)	2·7	0·8
Poly(A,C)	[c]		*27 750*	0·06	(25 600)	24 390	*23 150*	(21 900)	0·06	0·6 (25%)	2·5	1·0
	[d]		*27 780*	0·12	25 640	24 330	*23 040*	(21 900)	0·09	0·6 (25%)	2·5	0·8
Poly(A,U)	[c]		*28 170*	0·04	25 580	24 330	*23 040*	(21 900)	0·04	—	2·5	1·0
	[d]		*27 780*	0·12	25 580	24 330	*23 040*	(22 000)	0·08	0·8 (25%)	2·7	0·7
Poly(G,C)	[c]	(30 100)	*28 090*	0·02	(26 100)	(24 500)	*23 530*	(22 300)	0·03	0·5 (20%)	1·5	1·5
	[d]	(29 950)	*27 930*	0·03	(26 100)	(24 500)	*23 530*	(22 320)	0·03	0·5 (15%)	1·5	1·0
Poly(C,U)	[c]		*28 100*	0·04			*22 950*		0·02	0·5	—	0·5
	[d]		*28 200*	0·02			*23 000*		0·01	0·5	—	0·5
Poly(A,G,C,U)	[c]		*27 800*	0·05	(25 800)	24 390	*23 360*	(22 000)	0·04	0·6 (25%)	2·4	0·8
	[d]		*27 600*	0·10	(25 640)	24 390	*23 200*	—	0·08	0·7 (25%)	2·4	0·8
Poly[(dA-T) . (dA-T)]	[c]		*28 000*	0·06			*21 790*		0·02	0·5	—	0·3
	[d]		*27 860*	0·09			*21 640*		0·03	0·5	—	0·3

[a,b] See the footnotes in Table I; [c] propylene glycol–0·1M phosphate buffer (pH 7) (1 : 1, v / v); [d] 0·05M sodium acetate, 0·25% glucose.

indicate that there are only slight differences between the room and low temperature emission as well as excitation spectra of these compounds. This result gives some justification for our attempt to compare various data obtained at room temperature on the one hand and at liquid nitrogen temperature on the other hand, even though the correlation was observed only for several dinucleoside phosphates. More data on room temperature luminescence obtained by recently proposed techniques[26-31] would be useful for the elucidation of the properties of excited states and their relation to the conformation of oligo- and polynucleotides in solution.

Low Temperature Luminescence Spectra of Trinucleoside Diphosphates

The data given in Table III show that all trinucleotide diphosphates which contain the sequence ···ApU··· yield the excimer peak as the only singlet emission. On the other hand, UpApA has the fluorescence similar to that of ApA with a shoulder corresponding to the excimer emission. Also ApApA has the position of fluorescence maximum identical with that of ApA. We did not observe the previously reported[32] gradual shift of the fluorescence band to lower energies with increasing length of the oligonucleotide chain, even though small red shift of phosphorescence took place at the same time. If the same media with pH 7 were used, the fluorescence maxima of adenine oligonucleotides up to poly(A) were always identical. There are no significant differences between phosphorescence spectra of various sequences of adenine and uracil residues in trinucleoside diphosphates as well as in dinucleoside phosphates.

Minor differences were observed between the fluorescence and phosphorescence quantum yields of various sequence isomers of the trinucleoside diphosphates studied; these values were always somewhat lower than those of the constituting dimers, *i.e.* ApU, UpA, or ApA.

Unfortunately, there is only limited information available on the conformation of trinucleoside diphosphates in solution[33,34]. However, in analogy to dinucleoside phosphates it can be supposed that the residues of the trinucleoside diphosphates consisting of the sequence ...ApU... can assume easily the conformation, which enables the formation of excimers; similarly to ApU the majority of such molecules exhibits fluorescence from excimer states. On the other hand, the probability of occurence of conformational conditions favorable for formation of excimers is low for UpApA.

Low Temperature Luminescence Spectra of Nucleotide Copolymers

The low temperature emission spectra of different polynucleotides, which represent copolymers of adenine, guanine, cytosine, and uracil in various combinations, were recorded in order to find out to what extent the quantum yields are affected by polymerization. From Table IV and Fig. 2 it can be seen that all nucleotide copolymers

studied exhibit expressive excimer bands, which in most cases represent the only singlet emission. In all cases the maxima of the excimer peaks lie very close to those of the corresponding dinucleoside phosphates. Also in analogy to dinucleoside phosphates, the phosphorescence band shows always features characteristic for emission from the residues with lowest lying triplet level. However, the whole phosphorescence band is slightly shifted to lower energies, similarly to the effect observed for homopolynucleotides[1,9].

Besides the component corresponding to the emitting residue, the phosphorescence decay of several nucleotide copolymers contains a short lived component with lifetime between 0·5 to 0·8 s. The nonexponential phosphorescence decay was observed earlier for homogeneous polynucleotides[1,9] and aggregates of purine derivatives[15] and is the characteristic property of triplet emission from longer mutually interacting stacks of chromophores. In the case of poly(A,G) the higher value of the short lived component indicates that most probably a small fraction of guanine residues phosphoresces, similarly as in ApG or GpA. It is not possible, however, to evaluate the eventual contribution of the residues with higher lying triplet levels in the case of the other two-base copolymers containing pyrimidine bases, the phosphorescence lifetimes of which lie[35] also close to 0·5 s.

The fluorescence quantum yields estimated in propylene glycol matrix remain for poly(A,C), poly(A,U), and poly(C,U) approximately at the same level as those for the corresponding dinucleoside phosphates; for poly(A,G) the fluorescence quantum yield increased, whereas for poly(G,C) decreased. The comparison of the fluorescence quantum yields φ_F of the oligonucleotides and polynucleotide of adenine and uracil shows that the small decrease of φ_P observed for trinucleoside diphosphates was insignificant. The phosphorescence quantum yields (φ_P) follow roughly the changes of fluorescence quantum yields; only for poly(A,C) a more pronounced decrease of φ_P takes place. It can be seen that the formation of longer nucleotide polymers does not lead generally to a decrease of quantum yields. The observed changes depend on the polynucleotide composition and might be dependent on conformational differences. The only strong reduction of quantum yields, as compared with the corresponding dinucleoside phosphates, is exhibited by poly(G,C).

The fluorescence quantum yield of a copolymer containing the four components of RNA, poly(A,G,C,U), has the value which could be expected on the basis of an average value calculated from quantum yields of the two-base copolymers. It is close to that of poly[d(A−T).d(A−T)] which, on the other hand, has quantum yield several times as high[36] as double stranded DNA. The phosphorescence quantum yield of poly(A,G,C,U) which originates on adenine residues is somewhat higher than that of poly[d(A−T).d(A−T)].

CONCLUSION

The obtained data on excimer emission from dinucleoside phosphates and the analogical copolymers support some conclusions on low temperature emission of DNA

made earlier[1]: The absence of any significant quenching of singlet emission in hydrogen bonded complex poly(G).poly(C) implies that the differences in quantum yields observed for DNA's with different base composition cannot be explained by total quenching of excitation energy on hydrogen bonded guanine–cytosine pairs. The variations in fluorescence quantum yields of dinucleotide phosphates as compared with the mixtures of corresponding nucleotides give evidence that the base sequence might represent an important factor determining quantum yields of excimer emission of DNA. Namely the longer sequences of guanine and cytosine residues should cause a significant decrease of quantum yield of DNA. This is implied by the low quantum yields of poly(G,C); on the other hand, poly(A,G,C,U) with random distribution of guanine and cytosine residues has quantum yields comparable with poly[d(A–T).d(A–T)].

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