INFLUENCE OF MATRIX COMPOSITION ON THE LOW TEMPERATURE EMISSION SPECTRA OF PURINE AND PYRIMIDINE DERIVATIVES*

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The low temperature emission spectra of adenine, guanine, their nucleosides and nucleotides, CMP, UMP and TMP have been obtained from various matrices in order to ascertain *a*) the influence of aggregation on the spectra and *b*) the extent of aggregation in different frozen solvents. It has been found that the ratio of phosphorescence to fluorescence decreases and that the phosphorescence decay of adenine and guanine derivatives is nonexponential. Besides the component characteristic for the lifetime of the monomeric solutes a short-lived component (τ = = 0·4—0·5 s) can be detected. The emission spectra of the aggregates resemble closely the spectra of the corresponding polynucleotides measured in matrices preventing the aggregation. Generally the emission spectra of purine and pyrimidine derivatives from frozen aqueous matrices should be considered as a mixture of emissions from the aggregated and monomeric solute molecules in varying proportions. The emission spectra characteristic for the monomeric state of the solutes have been obtained from matrices containing 0·2—0·3% glucose and $5 \cdot 10^{-3}$M or higher concentration of sodium acetate or citrate.

From the recent studies on the emission spectra of polynucleotides and their components[1–7] it is apparent that their low temperature spectra can be significantly influenced by the solvent system.

Hélène[4] investigated phosphorescence spectra of purine and pyrimidine nucleosides from matrices formed by freezing the solutions in pure water. Under these conditions the monomeric nucleosides form aggregates in which chromophore interactions similar to those occurring in nucleotide polymers take place. This results in a great similarity between the low temperature emission spectra of nucleoside aggregates and of polynucleotides; the latter being measured, however, under conditions not causing aggregation[6]. The aggregation can be disturbed if salts, alcohols, or substances with hydrogen-bonding potential are added[4,8,9]. While the pure aqueous matrix represents one extreme, the second one would be represented by a solvent which should not interact with the solute and modify the chromophore interactions upon freezing. The nature of polynucleotides limits the choice of the solvent. The type of solvent used most frequently in polynucleotide emission spectroscopy was the mixture of aqueous buffered solutions with a polyalcohol (the 1 : 1 mixture with ethylene glycol[2,3,10,11], propylene glycol[5,7], or 95% glycerol[1]). In con-

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trast to the opaque matrix which can be obtained by freezing of salt containing aqueous solutions, these matrices yield transparent homogeneous glasses which are more suitable from the technical point of view. However, while the polyalcohol–water mixture is convenient medium for the study of monomeric polynucleotide components, its use in the studies of polynucleotides is less unambiguous. In the alcoholic solutions polynucleotides become denatured at room temperature due to a decrease of hydrophobic interactions[12–14]. Even though it was shown[11] that the hypochromicity can be partially restorted by cooling to −60°C, there is still lack of information on the polynucleotide conformation under these conditions. The stronger base–solvent interactions (as compared with purely aqueous solutions) will decrease the base–base interactions, this effect being more pronounced for ordered conformations of single stranded polynucleotides[15]. In fact, a significant difference was observed between the emission spectra of single stranded polyadenylic acid measured in the ethylene glycol–water (1 : 1) mixture and in aqueous buffered solution[6], indicating the presence of more extent base–base interactions in the latter type of matrix.

In order to obtain information on the extent of aggregation in various media, the aggregation of purine and pyrimidine derivatives is examined in the present paper as a function of small changes in the matrix composition and of several other factors. In particular the changes in the fluorescence spectrum have been followed, because the shift of fluorescence maximum and the appearance of an excimer emission are used as a sensitive indicator of the chromophore interactions[5,12,16]. An attempt is also made to find a suitable solvent system which upon freezing should cause minimum aggregation of the monomeric base derivatives, but should secure intact polynucleotide structure with undisturbed base–base interactions.

EXPERIMENTAL

The purine and pyrimidine derivatives (Calbiochem, Los Angeles, California) were of A grade. Ethylene glycol (Mallinckrodt Chemical Works, St. Louis, Illinois) and propylene glycol (Hartman-Leddon Co., Philadelphia, Pennsylvania) exhibited no emission at liquid nitrogen temperature and were used without purification. For the preparation of all solutions bidistilled freshly boiled water was used. The samples in quartz capillaries 2 mm in diameter were frozen by immersion in liquid nitrogen. The emission spectra were recorded with Aminco-Keirs spectrofluorimeter at liquid-nitrogen temperature. The excitation wavelength was taken close to the lowest absorption maximum. For most of the substances it was 260 nm (38460 cm^{-1}); guanine and its derivatives were excited at 255 nm (39220 cm^{-1}) and 270 nm (37040 cm^{-1}). The spectra were corrected for the sensitivity of the detector system and plotted in energy scale. The spectra are shown on the figures in a scale corresponding to the respective quantum yields. At higher energies the total emission or its fluorescence part are drawn; the phosphorescence part of spectrum is always shown separately. The ratio of phosphorescence to fluorescence (P/F) was determined from the areas under the respective parts of the spectrum. Phosphorescence spectrum was separated by means of a rotating shutter and its decay was analyzed from the semilogarithmic plot of phosphorescence intensity *vs* time. Since the aqueous solutions formed opaque matrices, the quantum yields were estimated only semiquantitatively relative to the quantum yield of the same substance in ethylene glycol–water matrix. The quantum yields in ethylene glycol–water (1 : 1, v : v) medium were compared with known quantum yields of purine and pyrimidine nucleotides measured under similar conditions[3].

RESULTS

Adenine, adenosine and AMP. Two types of additives (representing electrolytes and hydrogen-bonding substances) were used in the study of the influence of matrix composition on the emission spectra of adenine and its derivatives, *viz.* NaCl and glucose. Their respective concentration range was chosen so that it included concentrations which are used usually in spectroscopy of nucleic acids. For comparison, spectra of the solutes in the monomeric form were obtained from the matrix of frozen mixture ethylene–glycol–H_2O (1 : 1, v : v). (Figs 1–6 and Table I).

As it has been shown by Hélène[4], freezing of nucleoside solutions in pure water causes aggregation of the solutes. The same effect can be observed also for nucleotides or the respective bases. The emission spectra of the aggregates of adenine, adenosine, and AMP in pure aqueous matrix are very similar, however, they differ in some details. For all three substances the fluorescence spectrum is structureless and shifted to lower energies as compared with the respective spectra of the monomeric forms. The magnitude of the shift is approximately the same for adenine and AMP (4600 and 4800 cm^{-1}, respectively) and somewhat lower for adenosine (about 3200 cm^{-1}). Similarly, the fluorescence quantum yield of adenine and AMP is reduced, while for adenosine remains approximately on the same level. From the shape and position of the fluorescence peaks and by analogy with the fluorescence spectra of dinucleotides[2,5], polynucleotides[2], or dinucleotide analogs[16] it can be concluded that these peaks correspond to the emission from excimer states.

The structure of the phosphorescence peak becomes less pronounced on aggregation, however, it can be observed if the solute concentration is higher than 10^{-3}M. Especially the AMP aggregates have the structure of phosphorescence peak relatively well resolved (Fig. 5*a*). Also the phosphorescence spectra are red-shifted. It is difficult to evaluate correctly the magnitude of the shift from the differences in positions of the maxima, because they can be influenced by the redistribution of intensity between different vibronic peaks. The shift of the phosphorescence blue edge upon aggregation is only several hundreds of cm^{-1}. The phosphorescence quantum yield is strongly decreased and the P/F ratio is lower than 1 for all three substances. The phosphorescence decay can be resolved into two components: Besides the component with lifetime close to the value of the monomer it contains 50–70% of a short-lived component with lifetime $\tau_1 = 0{\cdot}4-0{\cdot}5$ s.

The addition of NaCl or glucose to the solutions in pure water prior to freezing disturbs the aggregation of the solutes[4,8,9]. With increasing concentration of these additives the emission spectrum characteristic for the monomeric form gradually becomes dominant. The decrease of the number of solute molecules which are in the aggregated form is accompanied by the decrease of the proportion and finally by the disappearance of the short-lived component of phosphorescence decay. The structure in phosphorescence spectra appears already at low glucose or NaCl concentrations

and the P/F ratio increases. In fluorescence spectra the intensity of the excimer peak also gradually decreases and the monomer emission increases. In some cases the emission from both excimer and monomer states can be distinguished. The small variations in the energies of the both types of fluorescence maxima can thus be explained as due to their mutual overlap.

From changes of the parameters followed (Figs 2, 4, 6) it becomes evident that the disaggregation effect depends on the nature of the solute as well as of the substance added. Depending on these factors (and also on some others which will be discussed below), there exists a range of matrices of different compositions, in which both monomeric and aggregated forms can contribute to the observed emission in varying proportions.

For example, AMP has the highest tendency to remain in the aggregated form. Even at high concentrations of NaCl or glucose the dominant fluorescence emission is from the excimer state, the P/F ratio does not increase to the value characteristic for monomer spectrum, and the short-lived component constitutes a substantial part of phosphorescence decay (Fig. 6). Only the simultaneous addition of 0·25% glucose and higher concentration of a more effective salt, sodium acetate, leads to more pronounced disaggregation. However, even under these conditions the excimer emission (which is usually the most sensitive indicator of the presence of the aggregated form) dominates the fluorescence spectrum. The spectrum characteristic for the monomeric form was obtained from the sodium acetate–glucose matrix if the concentration of AMP was decreased

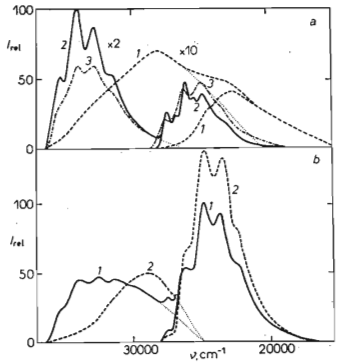

FIG. 1

Emission Spectra of Adenine in Different Matrices at 77 K (excitation wavelength 260 nm)

a $2 \cdot 10^{-4}$M adenine in pure water (*1*), ethylene glycol–water (1 : 1, v : v) (*2*), and $5 \cdot 10^{-5}$M adenine in $5 \cdot 10^{-2}$M sodium acetate–0·25% glucose (*3*); (*b*) $2 \cdot 10^{-4}$M adenine in aqueous solutions containing 0·4% glucose (*1*) and $5 \cdot 10^{-2}$M-NaCl (*2*).

to $5 \cdot 10^{-5}$M (Fig. 5). Even though the adenine aggregates are disturbed more easily than those of AMP, it is also necessary to use the combination of salt and glucose for the complete disaggregation (Fig. 2). The P/F ratio, which is about 0·4 for the aggregated form (in pure ice matrix) as well as for the monomeric form (in ethylene glycol–H_2O matrix) increases to values higher than 1 after the addition of salts or glucose. This increase can be attributed to a rise of phosphorescence quantum yield. Only the decrease of adenine concentration in the matrix containing both sodium acetate and glucose leads to the decrease of phosphorescence quantum yield; the P/F ratio then approaches the value 0·4 (Fig. 1*a*). The aggregates of adenosine are disturbed already by very low concentration of glucose and by medium concentration of NaCl (Fig. 4). Again the complete disaggregation is observed if glucose and an organic salt are added simultaneously.

The obtained results (Figs 2, 4, 6) indicate that in comparable concentration (0·2% glucose solution corresponds approximately to 10^{-2}M solution) glucose is more effective aggregation disturbant than sodium chloride. For example, the short-lived component of the adenosine phosphorescence decay can be detected (as an admixture

TABLE I

Emission Spectral Data of Adenine, Adenosine, and AMP in Various Matrices at 77 K

Matrix[a]	Fluorescence, max. cm^{-1}		Phosphorescence cm^{-1}		Lifetime[b] τ_2 s	P/F	Quantum yield	
	monomer	excimer	blue edge	max.			F	P
				adenine				
H_2O	—	28 090	27 200	22 730	2·3	0·4	0·019	0·007
NaG	32 570	—	28 200	24 940	2·7	1·1	0·15	0·16
EG-H_2O	34 010	—	28 200	26 110	2·3	0·4	0·06	0·025
				adenosine				
H_2O	—	28 740	25 700	22 990	2·7	0·2	0·03	0·01
NaG	30 670	—	27 000	24 750	2·8	1·3	0·04	0·05
EG-H_2O	31 850	—	27 000	25 000	2·8	1·7	0·01	0·02
				AMP				
H_2O	—	27 620	26 300	22 730	2·0	0·4	0·006	0·002
NaG	31 400	(29 000)[c]	26 700	24 510	2·8	1·0	0·015	0·016
EG-H_2O	31 850	—	27 030	24 940	2·7	1·1	0·01	0·013

[a] NaG $5 \cdot 10^{-2}$M sodium acetate with 0·25% glucose; EG-H_2O ethylene glycol–water (1 : 1, v : v). [b] Lifetime τ_1 (in parenthesis the relative proportion of the short-lived component is given) for adenine 0·5 s (40%), for adenosine 0·5 s (60%), for AMP 0·4 s (60%). [c] The respective singlet transition forms a shoulder in the spectrum.

constituting several per cent) at the highest NaCl concentrations used, while it disappears completely at lowest glucose concentration.

Exploiting the spectral changes characterizing the disaggregation process, we tested also the disaggregation effect of some other substances on adenosine samples. Urea, ethylene glycol, and propylene glycol have the effect similar to glucose in the same concentration range. Organic anions, *e.g.* acetate or citrate have higher effect than chloride ions. On the other hand, phosphates or sulphates are less effective. The alkali halides of lower molecular weight (exhibiting no external heavy atom effect) disturb the aggregation of adenosine in the order $NaF < LiF < KCl < LiCl \approx NaCl$.

As it has been already mentioned above, the extent of aggregation depends, under otherwise identical conditions, on solute concentration. The influence of concentration on spectra of AMP is shown on Fig. 5. Figs 7 and 8 illustrate the variations of adeno-

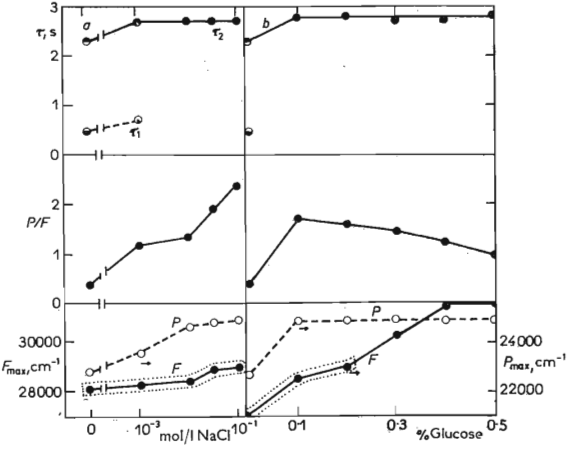

FIG. 2

Changes in Positions of Fluorescence (*F*) and Phosphorescence (*P*) Maxima, Phosphorescence Decay Times (τ_1 and τ_2) and *P*/*F* Ratio of Adenine Emission Spectra (concentration $2 \cdot 10^{-4}$M) at 77 K Obtained from Aqueous Matrices with Increasing Concentration of (*a*) Sodium Chloride and (*b*) Glucose

The dark segments on the points corresponding to the two components of phosphorescence decay indicate the approximate proportion of the respective components. The hatched part of the curve *F* indicates the dominance of the excimer emission. The arrows denote the appearance of structure in fluorescence and phosphorescence spectra.

sine spectra with increasing solute concentration: in the examined concentration region the aggregation of adenosine increases with concentration.

Also the ionization of solute molecules can increase the tendency to form aggregates. In aqueous matrices containing $5 . 10^{-2}$M sodium acetate and 0·25% glucose (*i.e.* under conditions in which the aggregation is suppressed) anions and cations of adenine, adenosine, and AMP yield fluorescence spectra with distinct excimer peak. In phosphorescence decay of protonized adenosine the short-lived component can be detected. A shoulder in the position of the excimer peak can be seen also on the spectra of protonized adenosine and AMP obtained from the matrix ethylene glycol–H_2O (1 : 1). The spectral changes due to aggregation should be distinguished from those, which are caused by the ionization of the solute molecule[17–19].

Other purine and pyridine base derivatives. The emission spectra in pure ice matrix, ethylene glycol–water (1 : 1), and sodium acetate–glucose matrices were recorded also for guanine, guanosine, GMP, CMP, UMP, and TMP. The spectral changes, which are dependent on the degree of aggregation of the substances listed above, are in qualitative agreement with those found for adenine and its derivatives (Table II).

Guanine derivatives are only partially aggregated in the pure ice matrix, as can be guessed from the simultaneous appearance of both monomer and excimer peaks in the fluorescence spectrum. The excimer peak can be identified by comparison with emission spectra of polyguany-

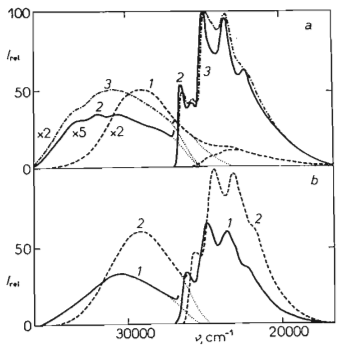

FIG. 3

Emission Spectra of Adenosine in Various Matrices at 77 K (excitation wavelength 260 nm) *a* $2 . 10^{-4}$M adenosine in pure water (*1*), ethylene glycol–water (1 : 1, v : v) (*2*), and $5 . 10^{-5}$M adenosine in $5 . 10^{-2}$M sodium acetate–0·25% glucose (*3*); *b* $2 . 10^{-4}$M adenosine in aqueous solutions containing 0·25% glucose (*1*) and $5 . 10^{-2}$M-NaCl (*2*).

TABLE II

Emission Spectral Data of Guanine, its Derivatives, CMP, UMP, and TMP in Various Matrices at 77 K

Matrix[a]	Fluorescence, max. cm^{-1}		Phosphorescence cm^{-1}		Lifetime[b] τ_2 s	P/F	Quantum yield	
	monomer	excimer	blue edge	max.			F	P
				guanine				
H_2O	30 580	28 170	28 500	23 640	1·5	0·4	0·04	0·02
NaG	30 860	—	28 500	24 100	1·5	0·7	0·11	0·08
EG-H_2O	30 960	(28 570)[c]	28 500	24 040	1·5	0·8	0·15	0·16
				guanosine				
H_2O	(29 700)[c]	28 570	27 600	22 880	1·4	0·3	0·05	0·02
NaG	30 120	—	28 200	24 940	1·4	1·5	0·29	0·43
EG-H_2O	30 120	—	28 500	25 130	1·4	1·7	0·14	0·24
				GMP				
H_2O	30 000	28 740	27 400	23 810	1·4	0·2	0·10	0·02
NaG	29 850	—	28 000	24 940	1·4	1·4	0·07	0·14
EG-H_2O	30 300	(28 570)[c]	28 000	25 130	1·4	1·1	0·13	0·14
				CMP				
H_2O	—	29 500	26 300	22 470	0·5	0·1	0·03	~0·003
HaG	31 050	—	28 000	24 330	0·7	0·4	0·05	0·02
EG-H_2O	31 150	—	27 900	23 980	0·8	0·03	0·05	~0·002
				UMP				
H_2O	—	30 400	26 670	22 470	0·4	0·07	0·02	~0·001
NaG	31 050	—	28 200	23 920	[d]	0·2	0·005	~0·001
EG-H_2O	31 250	—	27 800	23 810	[d]	0·06	0·01	~0·001
				TMP				
H_2O	—	29 940	25 200	21 740	0·4	0·05	0·08	~0·004
NaG	30 580	—	26 300	22 220	0·4	0·03	0·17	~0·005
EG-H_2O	30 580	—	26 300	22 320	0·5	0·03	0·16	~0·005

[a,c] See Table I. [b] lifetime τ_1 (in parenthesis the relative proportion of the short-lived component is given) for guanine 0·4 s (10%), for guanosine 0·5 s (70%), for GMP 0·5 s (50%). [d] Due to low phosphorescence quantum yield the lifetime could not be estimated.

lic acid[20]: The fluorescence spectrum of polyguanylic acid in all three considered matrices consists of only one broad peak with maximum at 28 500—28 900 cm^{-1}. The lowest degree of aggregation exhibits guanine; the short-lived component of its phosphorescence decay makes 10% of the total intensity. The P/F ratio as well as the total quantum yield are always decreased in the aggregated form. The spectra obtained from the sodium acetate–glucose matrix are close to those found in the ethylene glycol–H_2O matrix. The only differences are in the values of the P/F ratio for GMP.

The spectra of pyrimidine nucleotides are affected by the matrix favoring aggregation less than the spectra of adenine and guanine derivatives. The red shift of fluorescence maximum is 1 500 cm^{-1} for CMP and 600 cm^{-1} for UMP and TMP. This value is substantially lower than the shift of 3 000 cm^{-1} observed[6] for poly (C). On the other hand, the red shift of phosphorescence blue edge is between 1 100—1 700 cm^{-1} for the three pyrimidine nucleotides, which is comparable with the shift observed for adenine derivatives. The very small shift of fluorescence maxima of UMP and TMP is most probably a result of overlapping of the excimer and monomer peaks. No shorter component is phosphorescence decay could be detected and also the emission yields in the ice matrix were similar to those obtained from the two other matrices. Only the P/F ratio of CMP in the sodium acetate–glucose matrix is increased.

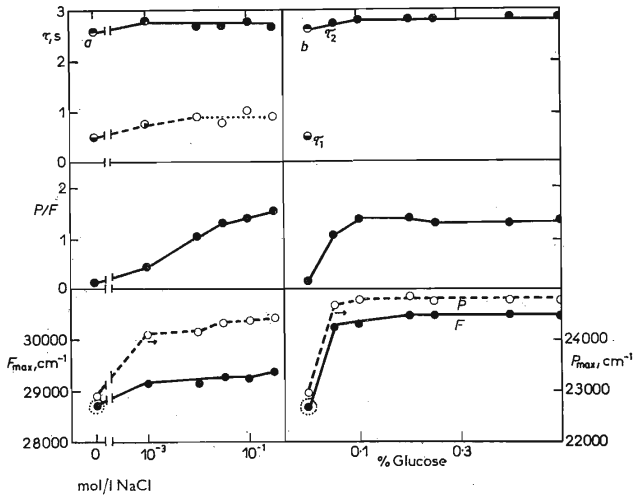

FIG. 4

Changes in Positions of Fluorescence (F) and Phosphorescence (P) Maxima, Phosphorescence Decay Times (τ_1 and τ_2), and P/F Ratio of Adenosine Emission Spectra (concentration 2 . 10^{-4}M) at 77 K Obtained from Aqueous Matrices with Increasing Concentration of (*a*) Sodium Chloride and (*b*) Glucose

The details are given in the legend to Fig. 2.

The obtained results indicate that the quanine derivatives as well as pyrimidine nucleotides have lower tendency to form aggregates than is exhibited by the derivatives of adenine.

DISCUSSION

Emission spectra of the aggregates. The aggregation of purine and pyrimidine derivatives which was described originally in pure ice matrices[4] is a phenomenon depending on the nature of the solute, its concentration and also on the composition of solvent (or on the presence of other solutes), which forms the matrix upon freezing. The basic features of the emission spectra of the aggregates are very similar to the spectra of nucleotide polymers or oligomers[5,6]. The exact structure of the aggregates is not known. However, their microcrystalline character[8,9] and the similarity of their emission properties with those of the polynucleotides indicate that both vertical stacking and horizontal interactions should be expected.

The emission spectral changes, by which the aggregates (obtained by freezing the solution in pure water) or the nucleotide polymers differ from the corresponding monomers are as follows:

a) Both fluorescence and phosphorescence spectra are red shifted[4]. In the fluorescence spectrum a new structureless peak appears at low energies, which corresponds

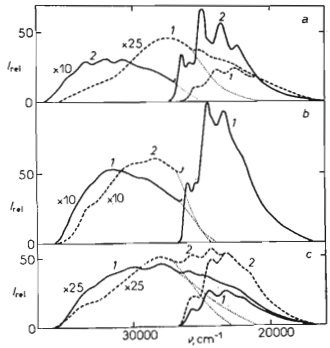

FIG. 5

Emission Spectra of AMP in Different Matrices at 77 K (excitation wavelength 260 nm)

a $4 \cdot 10^{-3}$M AMP in pure water (*1*) and ethylene glycol–water (1 : 1, v : v) (*2*); *b* $5 \cdot 10^{-5}$M (*1*) and $4 \cdot 10^{-3}$M (*2*) AMP in $5 \cdot 10^{-2}$M sodium acetate–0·25% glucose; *c* $4 \cdot 10^{-4}$M AMP in aqueous solutions containing 0·4% glucose (*1*) and 10^{-1}M-NaCl (*2*).

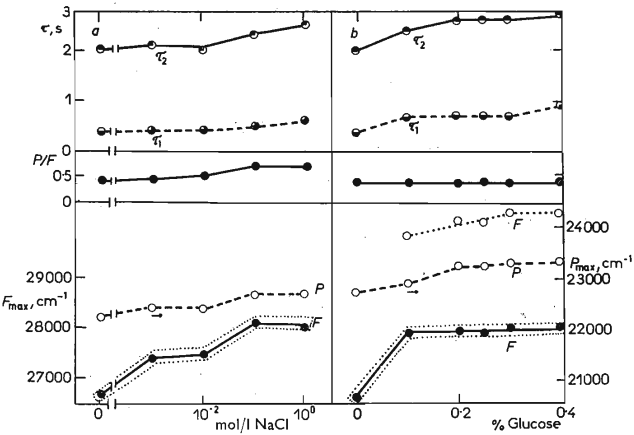

FIG. 6

Changes in Positions of Fluorescence (F) and Phosphorescence (P) Maxima, Phosphorescence Decay Times (τ_1 and τ_2), and P/F Ratio of AMP Emission Spectra (concentration $4 \cdot 10^{-4}$M) at 77 K Obtained from Aqueous Matrices with Increasing Concentration of (*a*) NaCl and (*b*) Glucose

In *b* the fluorescence spectrum contains two well resolved maxima, corresponding to emission from excimer (———) and monomer (·····). Other details see Fig. 2.

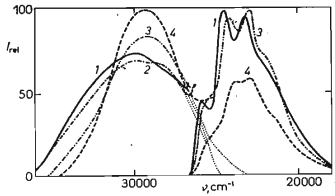

FIG. 7

Emission Spectra of Different Concentrations of Adenosine in Aqueous Matrix Containing 10^{-3}M Sodium Acetate at 77 K (excitation wavelength 260 nm)

$5 \cdot 10^{-6}$M (*1*), $2{\cdot}5 \cdot 10^{-5}$M (*2*)(the phosphorescence spectrum is identical with the curve (*1*)), $5 \cdot 10^{-5}$M (*3*), and $2 \cdot 10^{-4}$M (*4*).

to the emission from excimer state. Analogically to polynucleotides or oligonucleotides[2,5] the presence of the excimer peak reflects the interactions of solute molecules in a complex, which is formed between two neighbouring molecules, one in the lowest excited state, the other in the ground state. The magnitudes of the shifts differ for individual compounds (Table I and II); however the excimer peaks for completely aggregated samples lie all in the region 27 500 – 29 000 cm^{-1}. Within the same region are located the excimer peaks of DNA[2,6] and of various combinations of dinucleotides[2,5]. The red shifts of fluorescence and phosphorescence cannot be correlated. The changes in the position of phosphorescence blue edge, which can be taken as corresponding to the shifts of the 0, 0 band of the $S_0 \leftarrow T_1$ transitions, are caused predominantly by changes in the electrostatic interactions of the solute molecules with their environment.

b) The adenine and guanine derivatives have a non-exponential phosphorescence decay in the aggregated state. In addition to the component characteristic for the non-aggregated solute a short-lived component with the lifetime of 0·4 – 0·5 s can be detected. This component constitutes 50 – 70% of phosphorescence intensity in the pure ice matrix and its proportion decreases gradually with the disturbation of the aggregation.

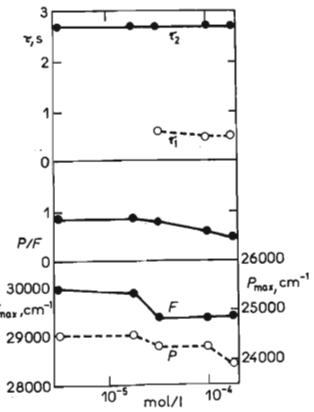

FIG. 8

Changes of the Spectral Parameters with Increasing Adenosine Concentration
The experimental conditions are the same as in Fig. 7; other details are given in the legend to Fig.2.

The short-lived component can thus be associated with the triplet emission from the mutually interacting solute molecules in the aggregates and reflects the increased rate of radiationless deactivations of the excited states in the aggregates; the purine polynucleotides behave similarly[6].* In accordance with that, the quantum yields of fluorescence and phosphorescence of the aggregates are mostly lower than those of the corresponding monomers. If the decreased quantum yield of phosphorescence of the aggregates is considered, it can be estimated that in pure ice matrix the phosphorescence radiative lifetime of in average 90% solute molecules corresponds to the short-lived component,

The P/F ratio, which can be taken as an approximate measure for the changes in intersystem crossing always decreases. For the quantum yield of intrinsic phosphorescence the following relation is valid

$$\Phi_P^0 = [k_{ISC}/(k_F + k_{IC}^S + k_{ISC})]\, k_P/(k_P + k_{IC}^T)$$

and the observed phosphorescence lifetime is proportional to Φ_P^0 (here k_F, k_P, k_{ISC}, k_{IC}^S, and k_{IC}^T are rate constants for fluorescence, phosphorescence, intersystem crossing, and internal conversions from the excited singlet and triplet states to the ground state, respectively). The observed decrease of phosphorescence quantum yield and its radiative lifetime can thus be also ascribed to increased rate of internal conversion from both excited singlet and triplet states.

The extent of aggregation in different matrices. The study of emission spectra of different purine and pyrimidine derivatives in various types of matrices at 77 K showed that the individual compounds can at least partially aggregate in a very broad range of matrix composition. The spectra obtained from aqueous matrices should be considered generally as a mixture of emissions from molecules in aggregated state and monomeric molecules in varying proportions. For the interpretation of these spectra it is therefore necessary to consider spectra obtained under extreme conditions (*i.e.* on the one hand from matrices in which the aggreation is complete and on the other hand from matrices in which the aggregation is absent) and evaluate their contributions to the observed spectrum.

In the same type of solvent system which forms the matrix upon freezing the aggregation of purine and pyrimidine derivatives increases with increasing solute ionization. Montenay-Garestier and Hélène[7] observed formation of relatively stable complex between cytidine and protonated cytidine in the vicinity of its pK in aqueous matrices containing either 0·1M-NaCl or up to 33% ethanol. Also in the solute concentration range (which is limited by their solubility) it has been observed

* The non-experimential phosphorescence decay was observed for polyguanylic acid in ethylene glycol–H_2O (1 : 1) matrix by Rahn and coworkers[21], who, however, suggested that the short-lived component might be associated with the isolated GMP molecules, while the long-lived one with the molecules in the aggregated form.

that purine derivatives aggregate in greater extent with increasing concentration. It could be supposed, however, that much higher solute concentrations might lead to a decrease of the aggregation.

As it has been mentioned already in this paper, the glass formed from the 1 : 1 mixture of a polyalcohol with aqueous buffer solution at 77 K represents a matrix in which most of the purine and pyrimidine derivatives are solely in the monomeric form. This type of solvent can, however, influence the base–base interactions in polynucleotides[12–14]. The results of the present study show that the aggregation of the purine and pyrimidine derivatives is effectively disturbed in a purely aqueous matrix containing $5 \cdot 10^{-3}-10^{-2}$M sodium acetate or citrate and 0·2–0·3% glucose. The spectra obtained from these matrices are very similar to those obtained from the polyalcohol–H_2O glasses and correspond to the emission from the non-aggregated solute molecules. The only disadvantage is the opacity of the samples which prevents the reliable determination of quantum yields. The addition of low concentration of glucose does not change the conformation and base–base interactions in polynucleotides at room temperature. Also their thermally induced helix-coil transitions remain unmodified (unpublished observation). The salt–glucose matrix thus represents a medium suitable for the study of conformation dependent changes in polynucleotide emission spectra.

The mechanism of the disaggregation process. The changes occurring when the emission spectra of purine and pyrimidine derivatives are measured from the matrices of pure ice have been explained by Hélène[4] as due to formation of microcrystalline aggregates of solutes in accordance with the results of Bruice and Butler[8] and Wang[9]. These authors also observed a disturbance of the aggregation if salts or alcohols were added to the solvent prior to freezing.

Apparently the extent to which different solutes are excluded from solution in the process of water crystallization depends, besides other factors, on the nature of the solute. The purine and pyrimidine derivatives exhibit relatively strong tendency to form aggregates (adenine derivatives higher than guanine derivatives and pyrimidine derivatives), which increases with solute concentration in the experimentally accessible concentration range. The efficient disaggregation effect is exerted by rather diverse types of "additional" solutes (electrolytes, hydrogen bonding substances, alcohols or polyalcohols), which have one common property, however, namely that they produce water structure breaking[22,23]. It is possible that upon freezing this effect is reflected by the increase of the total volume of regions containing the excluded solutes, which thus become more diluted. This conclusion is supported by the observation that various salts disturb the aggregation with widely differing effectivity. Good correlation has been found between the disaggregation effectiveness and the water structure breaking activity[23] for alkali halides.

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