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6-Thioguanine Luminescence Probe to Study DNA and Low-Molecular-Weight Systems

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6-Thioguanine, an antitumor drug, has been tested as a luminescence probe to study DNA and cryoprotector solutions at temperatures between 4.2 and 273 K. The electronic structure of the tautomeric and ionic forms of 6-thioguanine is studied comprehensively both theoretically and experimentally. An excited-state diagram of 6-thioguanine N9H tautomer is proposed. The temperature behavior of 6-thioguanosine is examined in different cryoprotector solutions and with different aggregate states of solvents. Structure and phase transitions in low-molecular-weight cryoprotectors (glycerol, ethanol, propanediol, DMSO) and their water solution are investigated in the 4.2–273 K temperature range. New structural transitions in propanediol–water solutions are found in the temperature interval 10–180 K. DNA solutions are investigated by using 6-thioguanine incorporated in DNA by the method of biosynthesis. Phosphorescence intensity curves for 6-thioguanosine in native DNA manifest peculiarities at 21, 64, 87, 140, 180, and 268 K.

KEY WORDS: 6-Thioguanine; DNA; phosphorescence probe; low temperatures cryoprotectors.

INTRODUCTION

The structure and dynamics of proteins can be successfully studied by using natural chromophores of protein molecules (tyrosine, tryptophan, phenylalanine).[1] Nucleic acids contain practically no bases with substantially divergent spectral properties. The Y-bases 2-thiouracil and 4-thiouracil (minor bases of some tRNAs) can be used as a luminescence probe.[2,3] The structure and the dynamics of DNA can be studied by using 6-thioguanine (6SG), which is of particular interest because of its capability for inserting into DNA instead of guanine.[4] 6-Thioguanine is an antitumor drug.[5] We have studied comprehensively UV absorption, CD, and luminescence spectra of 6-thioguanine and its derivatives to gain better insight into the spectroscopic properties of 6SG and its nucleoside 6-thioguanosine (6SGR).[6–8] The experimental data are supplemented with theoretical results obtained by CNDO/S methods.[6–8] To solve the question as to wheather 6SG can be used as a luminescence probe we conducted comprehensive investigations of its luminescence spectra at various pH, in different solvents and DNAs, at various temperatures and different aggregate states of solvents. Luminescence thermograms of 6SG in low-molecular-weight alcohols (ethanol, glycerol, propanediol) as well as in DMSO and DNA aqueous solutions are examined.

EXPERIMENTAL

6-Thioguanine (6SG) and 6-thioguanosine (6SGR) and their derivatives were synthesized at the Institute of Organic Synthesis, Latvian Academy of Sciences (6SGR is a fixed N9H tautomer of 6SG). 6-Thiopurine-riboside

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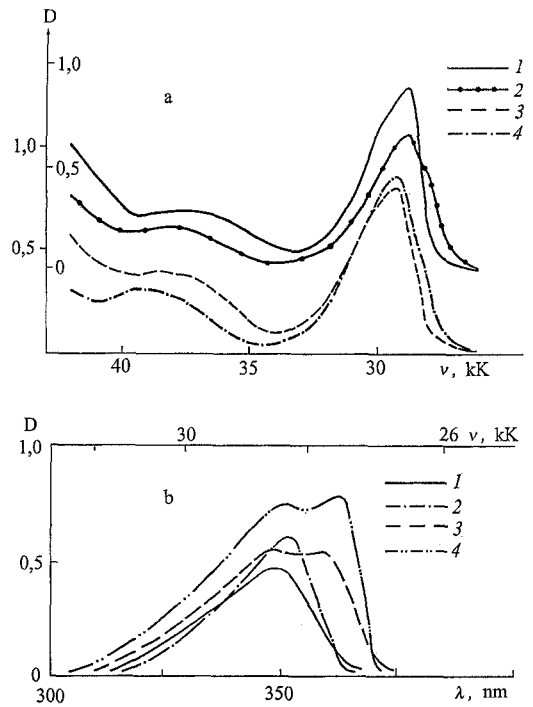

Fig. 1. (a) UV absorption spectra of 6SGR and 6SG (pH 5.5). (1) 6SGR in ethanol, (2) 6SG in ethanol, (3) 6SGR in water, (4) 6SG in water. Concentration is 10^{-5} *M*. (b) UV absorption spectra of 6SG and 6SGR in ethanol at 77 and 293 K. (1) 6SGR at 77 K, (2) 6SGR at 293 K, (3) 6SG at 77 K, (4) 6SG at 293 K. Concentration is 10^{-4} *M*, length of the optical path in cuvette is 1 mm.

was purchased from Serva Co. The alcohols [ethanol, glycerol, propanediol (PD), dioxane, and dimethyl sulfoxide (DMSO)] were preliminarily distilled and dried. DNA from *Escherichia coli* was used, into which 6SG was introduced by the biosynthesis method.[4] The concentrations were 10^{-5} *M* 6SGR in alcohols and 10^{-3} *M* DNA in solution. The relative 6SG concentration in DNA was one nucleotide with 6SG per 600 major nucleotides. The DNA solution contained 0.015 *M* NaCl and 0.0015 *M* sodium citrate. The DNA molecular weight was 5×10^6 Da. To obtain denaturated DNA, its initial solution was kept in boiling water bath for 30 min and then cooled to 4°C.

DNA films colored with 6-thiopurine-riboside were also studied. The spectral features of the latter are close to those of 6SG.[9] The preliminary dried film was kept in an atmosphere of KCl aqueous solution of the required concentration, which provided the necessary humidity (80%). The UV absorption spectra at 293 K were recorded with a Hitachi spectrophotometer. Circular dichroism spectra were taken with a Jobin Ivon dichrograph.

To prepare experimental samples, a special cuvette (volume 0.3 ml) with the prepared solution was quickly dipped into liquid nitrogen. Then the sample was cooled to liquid helium temperature. The rate of cooling was 6–9 K/min at 4.2–77 K, and that of heating was 0.7–1 K/min in the range 4.2–273 K. The temperatures were measured with a differential cuprum–constantan thermocouple.

The phosphorescence spectra and the temperature dependence of the probe phosphorescence intensity were obtained using a laboratory luminescence setup incorporating a nitrogen–helium cryostat and a microcomputer. When measuring the temperature dependence of probe emission, the exciting light wavelength was 350 nm and the observation wavelength was 483 nm. The sample transparency was studied using the same setup at λ = 483 nm; an incandescent lamp was used as a light source.

RESULTS AND DISCUSSION

Spectroscopy and Electronic Structure of 6-Thioguanine

To study the spectroscopic properties and electronic structure of 6-thioguanine, we investigated in detail UV, CD, and luminescence spectra of solutions of 6SG and its derivatives that simulate its tautomeric forms: 6-thioguanosine (6SGR), dietoxy-2,9-ethyl-6-thiopurine (6SPR), diethoxy-2,9-ethyl-6-methyl-mercapto-guanosine (6MeSGR), and 6-methyl-mercapto-purine-riboside (6MeSPR). Spectrophotometric titration was made, and ionization constants were determined for 6SG. Energies of excited states and elements of the electronic structure of 6SG in the ground and the excited states were calculated by the CNDO/S method for different ionic and tautomeric forms of 6SG.

The UV absorption and luminescence spectra of 6SG as compared to those of guanine (G)[10,11] are distinguished by low-frequency spectral line displacement by 4–8 kK, in spite of its ionic and tautomeric composition, permitting 6SG to be used as a luminescence probe when studying DNA (Figs. 1 and 2, Table I). The absorption and luminescence spectra of 6SG derivatives as well as those of 4SU[12] which have sulfur substituents (6MeSGR, 6MeSPR, 4MeSU) are substantially displaced (by 3–4 kK) to the high-frequency region with respect to the spectra of compounds with no sulfur substituents.

The absorption spectra of 6SG in aqueous solution and ethanol at 293 K and luminescence spectra in eth-

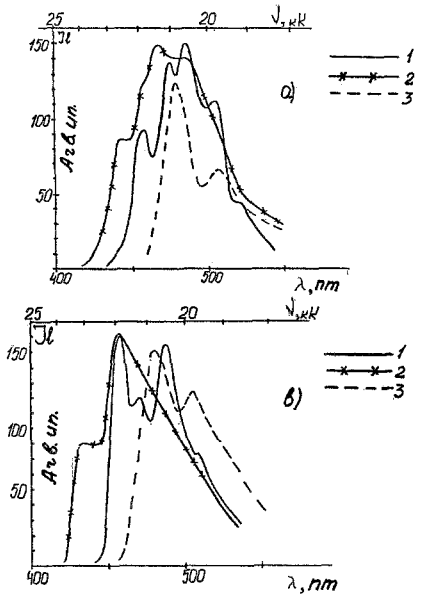

Fig. 2. Phosphorescence spectra of 6SG (a) and 6SGR (b) in ethanol (77 K) at various pH: (1) pH 6.5, (2) pH 10, (3) pH 1.

Table I. Positions of Maxima of UV Absorption Spectra (ν_{abs}^{max}) (293 K), Molar Extinction Coefficient (ε), and First Maximum in Phosphorescence Spectra ($\nu_{phos}^{0\text{-}0}$) (77 K) of Different Forms of 6SG and Its Derivatives

Molecule form	pH	ν_{abs}^{max} (kK)	$\varepsilon \times 10^{-3}$	$\nu_{phos}^{0\text{-}0}$ (kK)
6SG	1	28.8	20.8	20.9
6SG	5	29.2	22.2	21.8
6SG	10	31.0	18.5	22.6
6SG	1 N NaOH	31.2	21.2	22.6
G	6	36.4	8.1	26.3
6SGR	1	28.7		20.8
6SGR	5	29.1		21.8
6SGR	12	31.6		23.2
6MeSGR	6	32.1		22.9
6SPR	1	30.5		
6SPR	6	30.9	18.8	22.0
6SPR	12	32.2		
6MeSPR	6	34.9		

anol at 77 K of neutral forms of 6SG and 6SGR (Figs. 1 and 2) are some what different in their shape (in particular, in half-width of the first UV absorption band and in the number of peaks in the luminescence spectra).

Our study on the UV absorption spectra of 6SG and 6SGR solutions in ethanol at 293 and 77 K reveals the existence of a long-wave absorption in the 6SG solutions within the 370-nm region which is not observed in 6SGR (Fig. 1b). Exploration into the excitation wave-

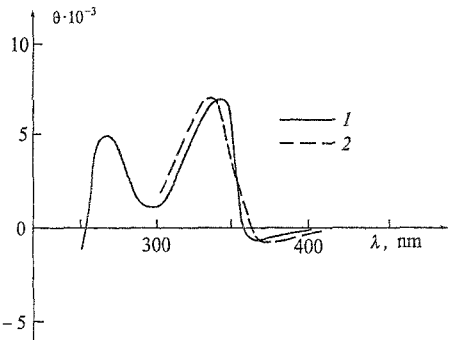

Fig. 3. Circular dichroism (CD) spectra of 6SGR (1) in ethanol, (2) in dioxane.

length dependence of the luminescence spectrum of 6SG demonstrates that at excitation λ = 365 nm the phosphorescence spectrum has a minimum number of peaks (at 477 and 502 nm), while at excitation λ = 355, 345, and 335 nm there appear new maxima (458 and 483 nm) in the luminescence spectrum which are typical of 6SGR, the latter being a fixed N9H tautomer of 6SG. The observation provides support for the existence of two tautomers of 6SG—N7H and N9H. It is known that in crystals 6SG exists as N7H tautomer.[13]

According to calculations of Ref. 14, the N7H tautomer, is the most energetically stable. But the large dipole moment (~8, D) of the tautomer N9H shifts essentially the tautomeric equilibrium in polar solvents to the tautomer N9H.

Data on essential differences in peak positions in the absorption and luminescence spectra of 6SGR and 6MeSGR as well as in the spectra of 4dSUR and 4VeSU, 6SPR and 6MeSpr, combined with X-ray data for 6SG crystals, permit the conclusion on the thione form of 6SG.

In addition to the intense band in the vicinity of 28.6 kK (350 nm), there also exists a low-intensity, long-wave negative band near 27 kK (370 nm) in the CD spectrum of the 6SGR neutral; form (Fig. 3). This band undergoes a bathochromic shift in dioxane (low polar solvent) indicating that it is a band of $n\pi^*$ type. This supposition is in an agreement with the quantum-chemical calculation data on excited-state energies (Table II) of the 6SG neutral form.

In alkaline media the UV absorption and luminescence spectra of 6SG and 6SPR shift to the high-frequency region (Table I, Fig. 2). The shift values as well as the luminescence spectrum shapes are different in 6SG and 6SGR, in agreement with the existence of two 6SG tautomers. At the same time in acid media the absorption and luminescence spectra of 6SG and 6SGR

Table II. Calculated (Calc) and Experimental (Exp) Excited-State Energy (E) and Oscillator Strength (f) of Different Ionic and Tautomeric Forms of 6SG

Form of molecule	$E_{S\pi\pi^*}$ (eV) Calc	Exp	f	$E_{T\pi\pi^*}$ (eV) Calc	Calc	Exp
Cation	3.29	3.56	0.50	2.43	2.12	2.59
Neutral N9H	3.68	3.61	0.47	3.01	2.71	2.70
Neutral N7H	3.67	3.40	0.43	3.19	2.73	2.59
Anion N9H	4.00	3.92	0.33	3.73	3.23	2.87
Anion N7H	4.09	—	0.32	3.93	3.26	2.80
Dianion	3.73	3.87	0.36	3.39	2.93	2.80

shift to a low-frequency region of the spectrum and become similar.

Analysis of the UV absorption and luminescence spectra (Fig. 2, Table I) of 6SG and its derivatives taken at various pH values permits us to make some suppositions. The similar shift direction for the first band in the UV absorption and luminescence spectra of 6SG and 6SGR (for the latter the deprotonation is possible only in position N1) and the similar shift direction during the neutral form–anion transition of 4dSUR (its deprotonation is also possible only via an unsubstituent nitrogen atom)[12] permit us to suggest that 6SG and 6SGR are deprotonated via the N1 atom. This correlates with the smaller charge value on N1 compared with the amino-group nitrogen (Table III). In addition to the similarity of the UV absorption and luminescence spectra of 6SG and 6SGR in the acid media, their simultaneous red shifts suggest that 6SG is protonated via N7 or N9, depending on whether the original neutral molecule of 6SG is N9H or N7H tautomer, respectively. On the whole, the analysis of the absorption and luminescence spectra of solutions of 6SG and its derivatives at various pH, compared with the spectroscopic data of guanine[11] and 4-thiouracil,[12] permits us to propose a scheme of acid–base equilibrium of 6SG (Fig. 4). As seen in Fig. 2 and Table I, the luminescence spectra of the ionic forms of 6SG display only phosphorescence, caused by a strong spin–orbital interaction in the sulfur atom.[15] A possible reason for this seems to arise from "the heavy atom effect" and the presence of close electronic levels of different orbital nature ($n\pi^*$ and $\pi\pi^*$ type; see the energy diagram below).

The phosphorescence spectra of all the forms of 6SG, 6SGR, and 6SPR have a pronounced vibrational structure in which a vibration $\nu = 1250 \pm 20$ cm^{-1} can be clearly recognized for neutral forms and cations, while the spectrum of guanine displays a predominant vibration $\nu = 1500$.[10] This is probably due to the different excitation localization by the molecules of guanine and 6SG (see below). The phosphorescense of the 6SG neutral form is likely to be of type because its decay time ($\tau \sim 32$ ms) is substantially larger than that of 4SU (200 μs), the phosphorescence of which is of $n\pi^*$ type.[16]

The high absorption intensity of the first UV absorption intense band ($\nu = 28.6$ kK) of the 6SG and 6SGR neutral forms and its hypsochromic shift in dioxane (Fig. 3) permit us to suggest that the above band is of $\pi\pi^*$ or a $1a_\pi$ type.

The energies of the excited states (Table II) and the elements of the electronic structure (charges on atoms, bond orders, spin density) (Table III) in the ground and the excited states were calculated for neutral and ionic forms of 6SG by the CNDO/S method. The sulfur atom contribution to the resonance integral was 14 eV and the single-center electron repulsion integral was taken equal to 7 eV. These parameters were optimized using the calculated data for a large group of sulfur-containing compounds (ionic and tautomeric forms of 4SU, 2SU, 2,4SU, etc.).[6–8]

Analysis of the calculated excited-state energies (Table II) reveals that they are in good agreement with experimental energies of electronic transitions. The calculations show that the lowest singlet state of the 6SG neutral form is of $n\pi^*$ type.

The comparison of the values of charges on atoms for guanine[11] and 6SG (Table III) reveals their significant similarity in the ground state. In particular, this permits the N9H tautomer of 6SG to introduce into DNA. The values of the net charges on atoms at the transitions into $S_{\pi\pi^*}$, $T_{\pi\pi^*}$, and $n\pi^*$ electronic states change essentially on C6 and S10 atoms. The largest change in the bond order is observed for the C∈S bond. This suggests that the excitation in the above states is localized on the C∈S fragment, rendering it the most photoreactive. Theoretical calculations reveal a significant decrease of the electronic density on the S10 atom and at the same time its increase on the C6 atom during the transition into the electronic excited states. The calculations also show that the dipole moment of the 6SG neutral molecule decreases in the excited states from 10.80 D in the ground state to 6.69 D in the $S_{\pi\pi^*}$ and 6.67 D in the $T_{\pi\pi^*}$ states respectively. According to the classification by Kasha and Rawls,[17] these bands have to be assigned to the electronic transitions of $1a_\pi$ type.

The decrease of the electronic density on the sulfur atom in low excited states suggests that H bonds in the 6SGua+Cyt pair decrease considerably on exposure of DNA to UV light ($\lambda = 350$ nm).

The calculation of the H-bond energy in the

Table III. Complete Charges Q in S_0, $S_{\pi\pi^*}$, $T_{\pi\pi^*}$, and $n\pi^*$ States, $\pi\pi^*$ Charges in S_0 (Q_0), Spin Density ρ in $T_{\pi\pi^*}$ State, and Bond Order P in S_0 and Its Changes in Lower $S_{\pi\pi^*}$, $T_{\pi\pi^*}$ and $n\pi^*$ States of Neutral Form of 6SG N9H Tautomer

Form		N1	C2	N3	C4	C5	C6	N7	C8	N9	S10	N11	
N9H	Q_0	−0.04	0.32	−0.34	0.13	−0.04	0.27	−0.22	0.11	−0.01	−0.49	−0.19	
	$Q_{S_{\pi\pi^*}}$	−0.09	0.33	−0.32	0.06	0.06	−0.20	−0.27	0.12	−0.01	0.00	−0.18	
	$Q_{T_{\pi\pi^*}}$	−0.08	0.34	−0.34	0.06	0.05	−0.20	−0.26	0.13	−0.02	0.01	−0.18	
	$Q_{n\pi^*}$	−0.10	0.32	−0.38	0.04	−0.01	−0.27	−0.25	0.08	−0.01	0.26	−0.19	
	q_0	1.65	0.83	1.40	0.96	1.09	0.75	1.26	0.96	1.62	1.65	1.84	
	ρ	0.12	0.02	0.11	0.19	0.10	0.51	0.05	0.09	0.01	0.81	0.01	
Form		1–2	1–6	2–3	2–11	3–4	4–5	4–9	5–6	5–7	6–10	7–8	8–9
N9H	P_0	0.47	0.43	0.69	0.44	0.45	0.63	0.48	0.47	0.48	0.64	0.77	0.51
	$\Delta P_{S_{\pi\pi^*}}$	0.00	−0.19	−0.02	0.01	−0.01	−0.08	−0.01	0.04	0.00	−0.38	−0.04	0.00
	$\Delta P_{T_{\pi\pi^*}}$	0.02	−0.17	−0.03	0.01	−0.02	−0.08	−0.02	0.03	0.02	−0.38	−0.05	0.01
	$\Delta P_{n\pi^*}$	0.01	−0.19	0.00	0.00	−0.06	0.02	−0.02	−0.05	0.01	−0.30	−0.03	0.01

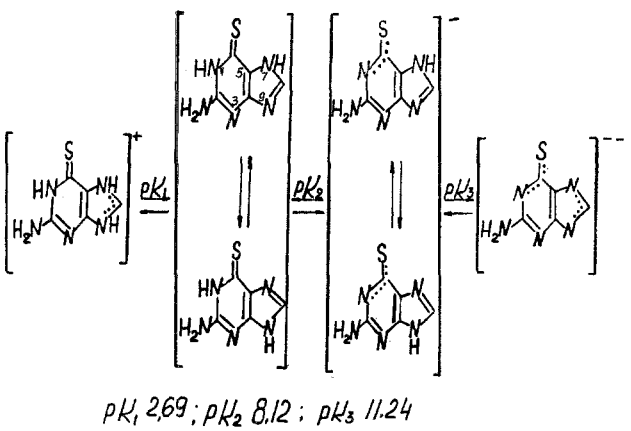

Fig. 4. Acid–base equilibrium of 6SG.

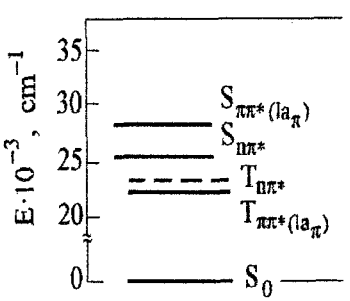

Fig. 5. Diagram of excited states of neutral form of 6SG (N9H tautomer).

6SGua+Cyt pair in the ground state shows that it decreases to −8.02 cal/mol compared with that of the H bonds of the canonical pair (−18.7 cal/mol).[18]

The spectroscopic and theoretical results considered here permit us to propose the diagram in Fig. 5 for the excited states of the 6SG neutral molecule (Fig. 5.).

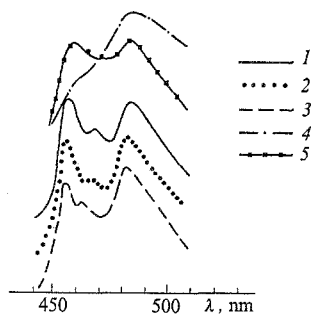

Fig. 6. Phosphorescence spectra of 6SGR in different solvents and DNA at 4.2 K. (1) Ethanol, (2) propanediol, (3) glycerol, (4) water, (5) native DNA in aqueous solution.

Thus, the spectroscopic and the calculated data, the diagrams for excited states of 6SG, the bathochromic shift of the UV absorption and luminescence spectra, the predominance of phosphorescence in the luminescence spectrum, and, finally, the ability of 6SG to incorporate into DNA instead of guanine suggest that 6-thioguanine can serve as a convenient phosphorescence probe to study structural changes in DNA solutions at temperatures between 4.2 and 273 K.

Influence of Environment and Temperature on Luminescence of 6-Thioguanosine

The luminescence spectra of 6SG and 6SGR were studied comprehensively in different solvents, at various temperatures in solvents of different aggregate states, and in different DNAs to resolve the question as to whether 6-thioguanine can be used as a phosphorescence probe.

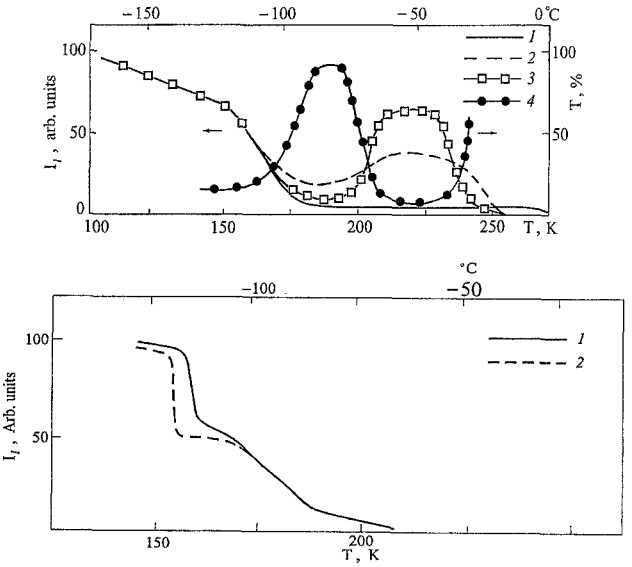

Fig. 7. (a) Temperature dependence of 6SGR phosphorescence intensity and transparency of propanediol (PD) solutions in water (at λ = 483 nm) on warming at a rate of 1 K/min. (1) 15% PD, (2) 40% PD, (3) 55% PD, (4) transparency of 55% PD solution. (b) Temperature dependence of 6SGR luminescence intensity in propanediol solutions on cooling at a rate of 1 K/min. PD concentration: (1) 15%, (2) 50%.

As seen from Fig. 6, there are two typical peaks in the vicinity of 458 and 483 nm in the phosphorescence spectra of the 6-thioguanine neutral form in ethanol. Moreover, the maxima of 6SGR in 20% DMSO are observed in the same spectral regions. These maxima shift to the low-frequency region (by 1 and 2 nm, respectively) as we pass from ethanol to propanediol and glycerol. The spectra of 6-thioguanine in cryoprotectors are similar. At the same time the phosphorescence spectrum of 6SGR in water differs essentially from the spectra in cryoprotectors in shape and position. This seems to be due to the aggregation of the 6-thioguanine molecules resulting from the formation of ice crystals and the displacement of the molecules studied into intercrystalline cavities.[16]

The phosphorescence spectrum of 6SG in DNA is similar in its form and position to those of the 6SGR neutral form in cryoprotectors, while it is considerably wider and shifted by 3 nm to the long-wave region with respect to that in ethanol. The spectrum of 6SG in denatured DNA is shifted to the long-wave region by another 3 nm. The form and the position of the phosphorescence spectra of 6-thioguanosine at various temperatures in solid states of cryoprotectors are similar. As the temperature is increased from 4.2 K to the onset of devitrification, the intensity of the first maximum in the region of 458 nm decreases as compared to that of the second one at 483 nm. The quantum yield of 6SG at 77 K is about 15%.[19] The decrease of the dipole moment in the excited states, as demonstrated by our theoretical calculations, suggested a negative solvatochromicity.[20] But the very weak luminescence (the quantum yield being about 0.1%) in the range of 380–450 nm with the maximum in the vicinity of 400–420 nm, observed after devitrification of the ethanol amorphous state, may be due to the fluorescence of 6SGR or impurities in ethanol. So the question of the position of the phosphorescence of 6SGR in the liquid solvent after the relaxation of the solvate shell needs further investigation.

To use 6-thioguanosine as a phosphorescent probe in studies of structural transitions in cryoprotectors, it is important to know the effect of the aggregate state of cryoprotectors and their solutions on the phosphorescence of 6-thioguanosine. In this respect, of particular interest is an investigation into the temperature dependence of the phosphorescence intensity of 6SGR in propanediol and its aqueous solutions on cooling and heating.

The temperature dependence of 6SGR phosphorescence intensity interesting shapes on heating in 15–60% propanediol solutions (Fig. 7a). It is useful to compare these curves with the temperature dependence of the transparency of the samples. One can observe a sharp decrease in the phosphorescence intensity and a concurrent increase in the transparency in the region of 160–175 K, perhaps due to devitrification of the amorphous states of the systems in question. Then, in the region of 190–200 K the phosphorescence intensity increases and at the same time the transparency decreases, perhaps due to the formation of ice crystals on heating.[21] Finally, the luminescence intensity decreases and the transparency increases again in the region of 230–250 K. In this case the sample becomes liquid. It is quite possible that the sharp decrease in the phosphorescence intensity of 6SGR on devitrification results from the significant displacement of the spectrum on the appearance of the liquid phase due to the relaxation of the solvate shell in the excited state (negative solvatochromicity is assumed). Further quenching of phosphorescence appears to be due to the significant shift of the potential energy curve in the triplet state in comparison to that in the ground state because of the changes in the electronic structure (and the dipole moment) in the excited state.[20]

The increase of the luminescence intensity in the range of 190–210 K on the appearance of ice crystals in the propanediol solutions may be due to the increase in the optical path of the exciting light in previously transparent samples. This is supported by the decrease of the

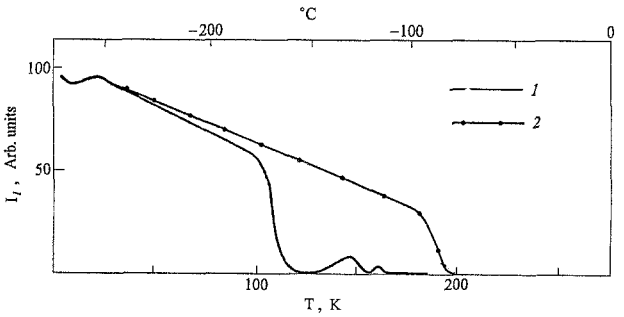

Fig. 8. Temperature dependences of 6SGR emission intensity in ethanol (1) and glycerol (2).

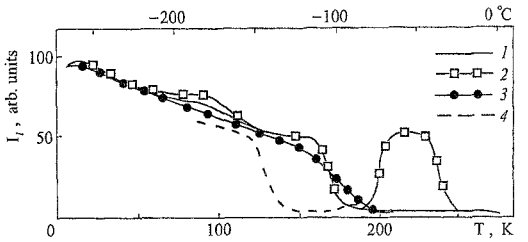

Fig. 9. Temperature dependence of 6SGR luminescence intensity in propanediol and DMSO solutions on warming at a rate of 1 K/min. (1) 15% Propanediol, (2) 50% propanediol, (3) 100% propanediol, (4) 15% DMSO.

emission intensity in the range of 230–250 K where the ice crystals melt.

Analysis of the normalized temperature dependence of the phosphorescence intensity of 6-thioguanosine in propanediol and its 15% and 50% solutions on cooling at a 1 K/min rate shows (Fig. 7b) that, in addition to the increase of the intensity in the region of 165–180 K due to the amorphous phase vitrification, one can also observe a sharp increase in the phosphorescence intensity in the vicinity of 155 K. At the same time the sample transparency decreases and the sample cell may be broken down. This results from the cracking of samples and is explained by the increase of the relative optical path for the exciting light in the cracked samples.

Thus, 6-thioguanosine (it is this material that we use in our experiment) can serve as a convenient phosphorescence probe in biophysical studies because its spectral properties vary with the medium pH, microenvironment, and temperature. Moreover, one can observe significant changes in the phosphorescence intensity on vitrification and devitrification of cryoprotectors, on formation and melting of ice crystals in propanediol–water mixtures.

Application of 6-Thioguanosine to Study Phase Transitions in Low-Molecular-Weight Cryoprotectors

Using the phosphorescence probe that we evaluated and studied, we examined structural transitions in glycerol and ethanol, propanediol, and their aqueous solutions as well as in aqueous solutions of DMSO in the temperature region from 4.2 to 273 K.

Analysis of the temperature dependence of the phosphorescence intensity of 6-thioguanosine in ethanol and glycerol (Fig. 8) on heating from 4.2 to 200 K shows that the phosphorescence intensity is reduced sharply in the region of 100–120 K for ethanol and 180–200 K for glycerol, perhaps due to devitrification of the amorphous states of ethanol and glycerol. This is in an agreement with calorimetric data.[22,23] The peculiar features observed in the region of 125–150 K for ethanol are consistent with calorimetric data on formation and melting of ethanol crystals.[24] As is also evident from Fig. 8, on heating from 20 K to the onset of devitrification the phosphorescence intensity of 6-thioguanosine decreases by a factor of 3/2 for ethanol and by a factor of 3 for glycerol. This is not accounted for by the decrease of the probe relative concentration in the solid solution, because the volume expansion coefficient for ethanol and glycerol glasses is ~0.0001.[22] Perhaps this is due to the increase in the intramolecular mobility of single fragments or the molecules on heating,[25] because the heat capacities of these substances increase with temperature.[22,23] This is supported by the observation of the gradual disappearance of a gap made by a laser at 5 K in the absorption spectrum of perylene in ethanol after heating of the solid solution at temperatures 25, 40, 77 K.[26]

In addition, we used 6-thioguanosine to study propanediol and its aqueous solutions at temperatures from 4.2 to 273 K. Analysis of the luminescence thermograms of the probe (Fig. 9) showed that for all the systems studied the maximum luminescence intensity was observed in the range 10–25 K rather than in the vicinity of 4.2 K. The curves of the temperature dependence of luminescence for the probe in pure propanediol and its aqueous solutions differ in position. The greatest differences are observed for 100% and 50% propanediol (PD). While the probe luminescence intensity in 100% PD decreases monotonously in the region from 10 to 150 K, the temperature dependence of the probe luminescence in 50% PD has a more complex behavior—its departure from the monotonous behavior is most pronounced at T = 90–100 K.

In aqueous solutions of PD the reduction in the temperature interval of the phosphorescence quenching

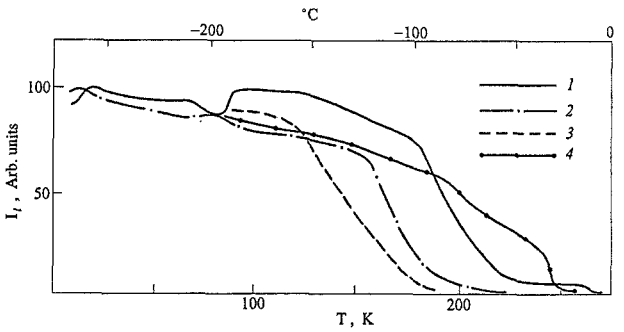

Fig. 10. Temperature dependence of emission of 6SGR incorporated into DNA under different conditions. (1) Aqueous solution, (2) 15% propanediol solution, (3) 15% DMSO solution, (4) the temperature dependence of 6SPR emission in the hydrated film of DNA (the probe was not incorporated into DNA).

is observed in the region from 160 to 180 K. Perhaps the increase and the decrease of the probe luminescence intensity in 50% PD in the region of 190–250 K (see above) are due to the secondary formation of ice crystals and their melting.

The analysis of the luminescence thermograms taken for the probe in 15%, 40%, 50%, 55%, 62% PD solutions showed good agreement between our results and calorimetric data[21] at temperatures from 140 to 273 K.

If we assume that the variation in the probe luminescence intensity is due to the structural changes in the solutions under consideration, it is quite possible that in the aqueous solutions of PD the structural changes (at least two) occur in the region from 25 to 160 K as well. Unfortunately, no calorimetric data for PD solutions are available for this temperature interval. No crystal phase has been observed for PD and its aqueous solutions. It also should be noted that the phase diagram of PD solutions proposed in Ref. 21 differs essentially from those of low- and high-molecular-weight compounds.[27–29] This suggests that the liquid–solid phase diagram proposed for aqueous solutions of PD[21] is incomplete.

The narrowing of the temperature interval of phosphorescence quenching in PD solutions compared with that in 100% PD in the region from 150 to 190 K, as well as the exothermal maximum of heat capacity observed in the vicinity of 170 K in,[30] suggest that in this temperature interval there occurs a melting of some ordered structure rather than the devitrification of the anisotropic amorphous phase as follows from the phase diagram in Ref. 21. Perhaps one should consider more carefully the observations of the liquid crystal phase in 45% PD solution.[21]

In our opinion, the existence of hydrophilic (OH) groups capable of hydration and hydrophobic (CH_2, CH_3) ones able to undergo hydrophobic interactions permits us to assume that liotropic liquid crystals may be formed in aqueous solutions of PD at temperatures below 170 K. These are supposed to consist of spherical, cylindrical, and lamellar structures, depending on cooling rate and PD concentration.[31]

Figure 9 also shows a decrease of the probe luminescence in the range from 100 to 120 K in DMSO solutions that correlates with the temperature interval in which the devitrification of the amorphous phase in the DMSO–water system takes place.[27]

It seems likely that the peculiarity in the region from 180 to 200 K is due to the formation and the melting of DMSO hydrate crystals.

Application of 6-Thioguanosine to Study DNA Solutions in the Temperature Range 4.2–273 K

Of significant interest is the study of DNA into which 6SG has been incorporated by the method of biosynthesis. We studied aqueous solutions of native and denaturated DNAs, DNAs in solutions with added PD and DMSO, as well as hydrated films of DNA containing no 6SG in its primary structure, but "colored" with 6-thiopurine-riboside.

The concentration of the 6SG probe in DNA was one molecule of nucleotide with 6SG per 600 canonical nucleotides. For the studied samples of DNA solutions with 6SG incorporated we observed a long-wave shoulder in the region from 300 to 350 nm (samples with D_{260} of ~10 optical units) of the UV absorption spectrum as well as a luminescence in the region from 450 to 550 nm at excitation in the vicinity of 350 nm.

The existence of luminescence of 6SG incorporated into native DNA and the relatively small differences in the luminescence intensity of 6SG in native and denaturated DNA (no more than by a factor of 1.5) show that, unlike the formation of the guanosine–cytidine pair,[32] the formation of 6-thioguanosine–cytidine pair in DNA does not result in luminescence quenching of nucleoside pairs.

Analysis of the luminescence thermograms of the probes (6SGR and 6SPR) shown in Fig. 10 demonstrates that the thermogram of the aqueous solutions of DNA has some peculiarities at T = 21, 65, 87, 145, 180, and 268 K. The most drastic variation in the probe luminescence intensity—the phosphorescence intensity fall—is observed in an interval from 180 to 220 K. For the DNA solutions with PD and DMSO added, the temperature interval of the luminescence fall is shifted to lower tem-

peratures typical of the devitrification of aqueous solutions with the concentrations shown in Fig. 10.[21,27]

In a DNA hydrated film in which the probe is located outside the DNA, the temperature interval of quenching is shifted to higher temperatures.

Analysis of the luminescence thermograms of the probe in a native DNA solution on cooling at a rate of 1 K/min shows that the increase in the luminescence intensity occurs within about the same temperature range as that in which the decrease of the intensity is seen in this sample on heating.[33] The thermograms of aqueous solutions of denatured DNA show a slight low-temperature displacement of the temperature interval of the probe luminescence quenching compared with that in the native DNA.

The similarity of the temperature interval for the decrease and the increase in the luminescence intensity of the probe on heating and cooling of DNA solutions, respectively, makes it impossible to attribute the decrease in the luminescence of the probe incorporated into DNA observed in the interval 180–220 K to the processes associated with ice crystallization—the transformation of cubic ice into hexagonal. It is known[34] that cubic ice is a metastable phase of ice I and is not formed on slow cooling of hexagonal ice.

The devitrification temperature of amorphous water is 135 K.[34] The above results for PD solutions (Fig. 7) show that the decrease of probe luminescence is observed when a liquid phase appears.

As shown in Fig. 10, the decrease in the luminescence intensity for the probe inside and that outside DNA occurs in the vicinity of 200 and 220 K, respectively. The increase in the temperature of the probe luminescence quenching is likely to be due to that of the temperature of the occurrence of liquid water (devitrification of water). This seems to be caused by the fact that this water is bound (it is a part of the DNA hydrate shell). It should be noted that, in accordance with Ref. 35, the devitrification of the protein hydrate shell takes place at 200–220 K.

The C=O fragment of guanine is known to be located in a major groove of DNA.[36] It is reasonable to suggest that the substitution of G by 6SG results in the C=S fragment on which the excitation is localized occurring in the major groove, too. It is known[36] that in the major groove of DNA three water molecules form H bonds with a cytidine amino group, a carbonyl group of guanosine, and an N7 atom of guanosine. It is quite possible that the sulfur atom when substituted for oxygen in the 6SG molecule forms an H bond with water. As the excitation in 6SG is localized on the C=S fragment, the photophysical processes in 6SG will be first affected by those which take place in the immediate vicinity of the C=S fragment.

It should be recalled that in the excited state a significant withdrawal of the electron density from the sulfur atom occurs and the molecule dipole moment decreases, resulting probably in a considerable weakening of the H bond of the group C=S followed by a displacement of the water molecule.

As is seen in Fig. 10, propanediol and DMSO added to the aqueous solutions of DNA, becoming a part of the DNA solvate shell at low temperatures because of the crystallization of most of the free water in the DNA solution at 250–273 K,[34] cause a decrease in the temperature interval of the probe luminescence quenching.

The above considerations suggest that the main reason for the probe luminescence quenching in DNA in the interval from 180 to 220 K is the devitrification of the DNA hydrate shell in the major groove. It is quite possible that the drastic increase of the probe luminescence intensity in the region of 87 K is due to the cracking of the samples (we observed a similar increase of the probe luminescence intensity on the cracking of the PD solution samples; Fig. 7b). The peculiarity in the vicinity of 268 K is likely to be due to the melting of aqueous solutions.

The reasons for the peculiarities observed in thermograms of aqueous solutions of native DNA at 21, 65, and 145 K are being studied. On the basis of the analysis of the luminescence spectrum of DNA with various concentrations of the probe of the macromolecule, we concluded that the energy, transfer to 6SG occurs on exposure of DNA to UV radiation.[33]

CONCLUSIONS

Our theoretical and experimental results permit us to propose a diagram of the excited states of the 6SG neutral molecule with the following sequence of electronic states: $S_0 < T_{\pi\pi^*(1a_\pi)} < T_{n\pi^*} < S_{n\pi^*} < S_{\pi\pi^*(1a_\pi)}$. These results showed also that 6-thioguanosine is a suitable probe to study the structure and dynamics of low- and high-molecular-weight systems. Structural transitions in propanediol–water solutions are found in the temperature interval from 10 to 180 K, using the probe studied (6-thioguanosine). Phosphorescence intensity curves for 6SG in native DNA manifest peculiarities at 21, 64, 87, 140, 180, and 268 K. The decrease of emission intensity in range 180–220 K probably is due to devitrification of the DNA hydrate shell.

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