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Characteristics of photoluminescence from ruthenium polypyridyl complexes incorporated into silk

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Abstract

Characteristics of photoluminescence from polypyridine Ru complexes incorporated into *Bombyx mori* silk fibroins with several structures have been investigated. The structures of the silk were α-helix, silk I, and silk II, that involve only intra-, both intra- and inter-, and only inter-molecular hydrogen bonds, respectively. Microenvironment in the silk was studied using photoluminescence probe. Maximum wave number of probes in silk was shifted to higher energy than that in water. The wave number decreased in the order, α-helix > silk I > silk II, suggesting that the polarity of the microenvironment is in the order, α-helix < silk I < silk II. The emission lifetime from the MLCT excited state in silk is three times as long as that in water, indicating that the probes exist in rigid microenvironment. There are two kinds of microenvironment around the incorporated probe because of the biexponential decay of the emission. The MLCT excited state lifetime was measured at various temperatures, and thermodynamic parameters such as the sum of radiative and nonradiative rate constants, and the activation energy from MLCT to d–d state were estimated to discuss the characteristics of the silk structures. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Bombyx mori silk; Photoluminescent probe; Microenvironment; Ruthenium complexes; Phosphorescence; Lifetime

1. Introduction

Bombyx mori silk fiber has been esteemed as a valuable material for cloths. Although synthetic fibers are now used all over the world, the silk is still keeping its position as a costly material. Interesting attempts have been carried out to utilize excellent features of the silk for oxygen sensors using photoluminescent probe molecules [1–3]. Oxygen can be measured by the emission intensity from the silk texture or film containing tris(2,2′-bipyridine)ruthenium(II) complex (abbreviated to $[Ru(bpy)_3]^{2+}$).

B. mori silk fibroin structures depending on the preparation have been reported in the latest studies [4–6]. They were classified in three types: α-helix type with intra-molecular hydrogen bonds [6], silk I type with both intra- and inter-molecular hydrogen bonds [4], and silk II type only with inter-molecular hydrogen bonds [5]. These structures can change to another one by treating with a certain organic solvent. It is interesting to obtain more information about microenvironment in such silk structures. A study of such microenvironment would be useful in developing a new device such as a photoluminescent probe sensor using silk in future. Utilization of photoluminescent probe is one of the promising approaches towards this direction.

We have selected $[Ru(bpy)_3]^{2+}$ and tris(1,10-phenanthroline)ruthenium (abbreviated to $[Ru(phen)_3]^{2+}$) as probes. The photochemical and photophysical properties of these complexes in various media have been studied well for the last four decades [7–13]. These probes have also been used for investigation of microenvironment in heterogeneous systems [12–15]. Assuming that the silk environment is a kind of medium for photoluminescent probes, the environment around the probe can be studied by the photoluminescent property.

In the present paper, we have investigated the microenvironment in the *B. mori* silk fibroin using these photoluminescent probes incorporated silks with various structures.

2. Experimental

2.1. Material

$[Ru(bpy)_3]Cl_2$ (bpy = 2,2′-bipyridine) and $[Ru(phen)_3]Cl_2$ (phen = 1,10-phenanthroline) were purchased from Aldrich Chemical Co. Inc.

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Silk I film was prepared as follows: degummed *B. mori* silk fibroin was dissolved in a 9 M LiBr aqueous solution, and the solution was shaken for 14 h, dialyzed against distilled water for 3 days, and then cast onto a pre-cleaned slide glass. It was dried under air for 3 days at 25°C. Silk II film was obtained by dipping the silk I film in methanol for a week. α-Helix film was made from silk I film by dissolving it in hexafluoro-2-propanol and was cast onto a pre-cleaned slide glass and then, dried under air at 25°C. The probe was introduced in a silk I film by the following procedures: a mixture of the probe and the silk solution was cast together onto a pre-cleaned slide glass and evaporated under air at 25°C. Other silk II and α-helix films incorporating a probe were prepared by using this silk I film with incorporated probe molecules.

2.2. Measurement

Emission spectra were measured with spectrofluorometers (Shimadzu RF-5300 with Hamamatsu photonics photomultiplier R928-08). In all the emission measurements, the sample membrane was placed in a quartz cell at 30° against the excitation light to minimize the effect of scattered light. The cell was degassed by introducing pure argon for 30 min. The emission decay was measured by a time-correlated single photon counting apparatus (Hitachi-Horiba NAES-550) equipped with a nitrogen lamp (10 atm) at 20°C.

3. Results and discussion

The three type silk structures are shown in Fig. 1 [6]. α-Helix, silk I and silk II are composed of intra-molecular hydrogen bonds, both intra- and inter-molecular hydrogen bonds, and only inter-molecular hydrogen bonds, respectively.

The absorption peak wavelength of $[Ru(bpy)_3]^{2+}$ in silk was almost the same as that in an aqueous solution. Fig. 2 shows corrected phosphorescence spectra of the probes incorporated in silks with various structures. Emission peaks of these probes in the silks are blue-shifted compared with that in water. The peak wave number decreased in the order, α-helix > silk I > silk II > aqueous solution. (see Table 1). It is well known that the excited state energy level

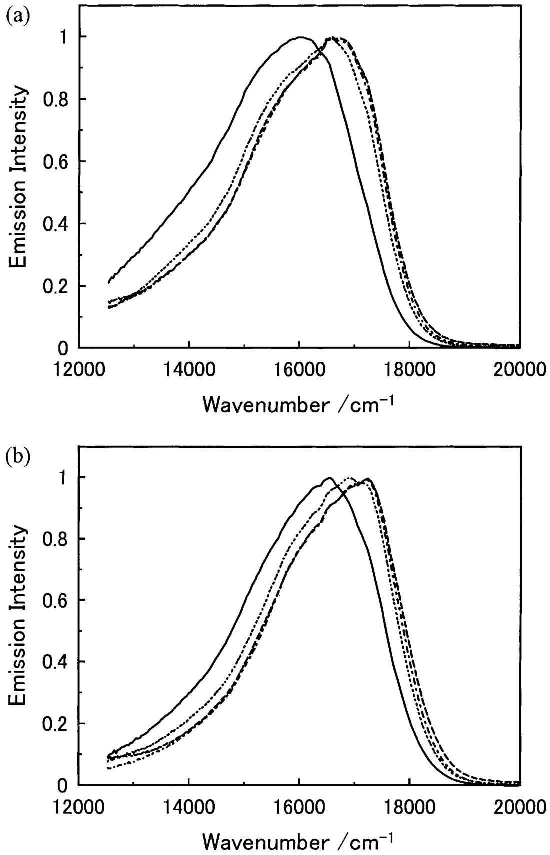

Fig. 2. Corrected emission spectra of (a) $[Ru(bpy)_3]^{2+}$ and (b) $[Ru(phen)_3]^{2+}$ at 293 K in various silk media and aqueous solution(-); (- - -) α-helix; (- · -) silk I; (· · ·) silk II.

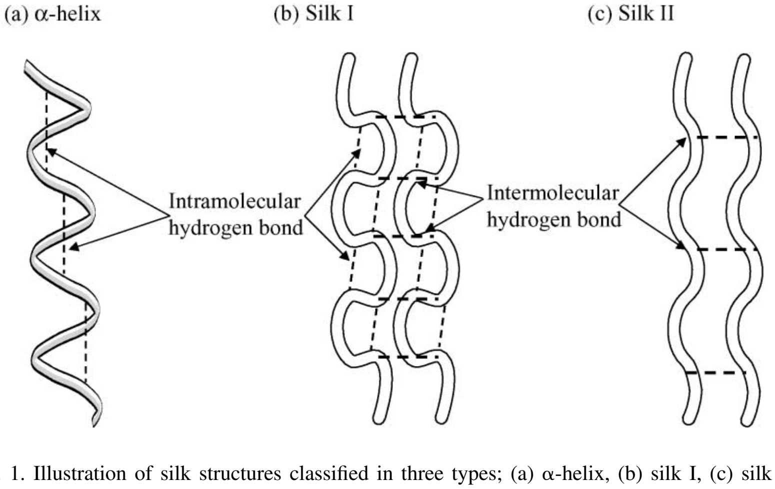

Fig. 1. Illustration of silk structures classified in three types; (a) α-helix, (b) silk I, (c) silk II.

Table 1
Dependence of emission properties of the probes on various silk media at 297 K

Media	$[Ru(bpy)_3]^{2+}$		$[Ru(phen)_3]^{2+}$	
	E_{em} (cm^{-1})	FWHM[a] (cm^{-1})	E_{em} (cm^{-1})	FWHM[a] (cm^{-1})
α-Helix	16,610	2790	17,240	2650
Silk I	16,580	2750	17,180	2560
Silk II	16,500	2820	16,890	2670
Water	15,950	3180	16,530	2800

[a] Full-width at half-maximum of the emission band.

of the probes such as $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ increases in non-polar media because MLCT in excited state of these probes has a more polarized structure than the ground state. These results show that the polarity of the microenvironment should be of the order of α-helix < silk I < silk II, which is rationalized by the patterns of the hydrogen bonds existing intra-, both intra- and inter, and only inter-molecularly, respectively. The emission peak of the probe in the silk II shows a lower energy gap between the ground and excited states than other silk media, which might be ascribable to the stabilization of the polarized MLCT excited state by the polar microenvironment of the silk II.

Another possible reason for these results is *luminescence rigidochromism* [13]. As mentioned above, the MLCT excited state is more polar in nature than the ground state. In a low viscosity solvent, the excited state would be quickly relaxed by rapid reorganization of the solvent, while in solid-like environment, the MLCT excited state would be fixed to unrelaxed state, so that the emission spectrum peak is shifted to higher energy in the silk.

Fig. 3 shows the phosphorescence decay curves of $[Ru(bpy)_3]^{2+}$ in silk II at various temperatures. The phosphorescence decay curve in all the silk media was multiexponential which has been often observed in heterogeneous systems [12,13]. The present decay curves could be fitted by biexponential decay function except $[Ru(phen)_3]^{2+}$ in α-helix. This suggests the existence of two kinds of microenvironments around the probe. Table 2 shows the lifetime

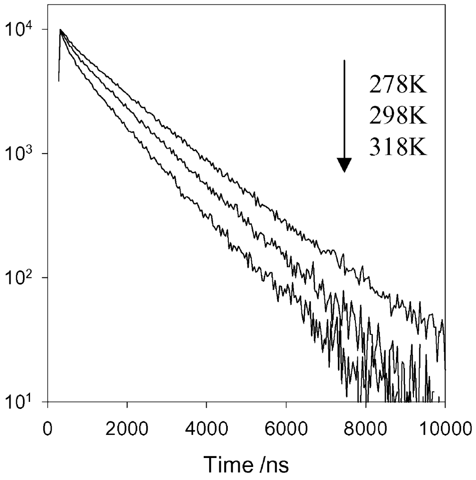

Fig. 3. Phosphorescence decay trace of $[Ru(bpy)_3]^{2+}$ in silk II at various temperatures. The concentration of $[Ru(bpy)_3]^{2+}$ in silk is 15 mmol dm^{-3}.

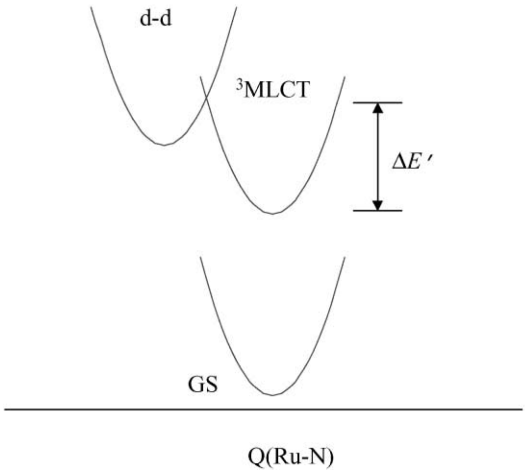

Fig. 4. Schematic energy diagram of the ground and excited states of $[Ru(bpy)_3]^{2+}$.

Table 2
Lifetime analysis of the luminescence decay of probes in silk media and in water at 293 K

Probe	Sample	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	χ^2
$[Ru(bpy)_3]^{2+}$	α-Helix	1750 (74.9%)	681(25.1%)	–	1.13
	Silk I	1890 (91.5%)	521 (8.5%)	–	1.05
	Silk II	1640 (79.7%)	699 (20.3%)	–	1.11
	Water	640[a]	–	–	
$[Ru(phen)_3]^{2+}$	α-Helix	3820 (42.2%)	1260 (44%)	309 (13.9%)	1.07
	Silk I	3990 (83.2%)	1330 (16.8%)	–	1.23
	Silk II	3490 (75.4%)	1150 (24.6%)	–	1.28
	Water	1170 (654)[b]	–	–	

[a] [6].
[b] [7].

Table 3
Analysis of Arrhenius plot for probes in silk media

	$[Ru(bpy)_3]^{2+}$			$[Ru(phen)_3]^{2+}$		
	α-Helix	Silk I	Silk II	α-Helix	Silk I	Silk II
$(k_r + k_{nr}) \times 10^5$ (s^{-1})	4.35	4.26	3.20	0.016	2.13	2.62
$k'^0 \times 10^9$	4.17	2.30	0.055	0.45	1.37	36.0
$\Delta E'$ (kJ mol^{-1})	23.7	23.8	12.1	16.6	23.9	31.9

analysis of $[Ru(bpy)_3]^{2+}$ in silk media at 293 K. The major lifetime is almost three times as long as that in water, suggesting that the former probe exists in a rigid environment that causes the *luminescence rigidochromism* [13], while the minor component (~600 ns) would be due to the microenvironment where the reorganization around the probe occurs easily since the lifetime is close to that in water. Similar behavior was also obtained for the $[Ru(phen)_3{}^{2+}]$.

The decay of $[Ru(bpy)_3]^{2+}$ is expressed by rate terms composed of three separated processes (see also Fig. 4) [11]: (1) emission (k_r) and radiationless deactivation (k_{nr}) from a low-lying MLCT state(s), and (2) thermal activation to a d–d state(s). It is informative for the present study to estimate the MLCT energy level because it depends on the microenvironment around the probe. The MLCT energy level relative to d–d level can be estimated by temperature dependence of the phosphorescence lifetime.

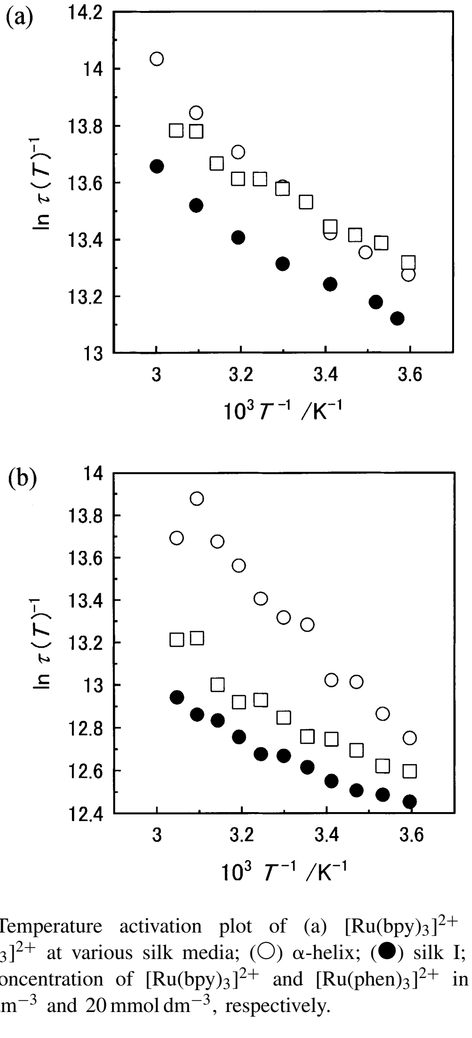

Fig. 5. Temperature activation plot of (a) $[Ru(bpy)_3]^{2+}$ and (b) $[Ru(phen)_3]^{2+}$ at various silk media; (○) α-helix; (●) silk I; (□) silk II. The concentration of $[Ru(bpy)_3]^{2+}$ and $[Ru(phen)_3]^{2+}$ in silk are 15 mmol dm^{-3} and 20 mmol dm^{-3}, respectively.

Fig. 5 shows the Arrhenius plot for the decay of the photoexcited state of the probes in the silk media. Thermodynamic constants are evaluated by nonlinear least-squares method with the following equation.

$$\ln\tau(T)^{-1} = k_r + k_{nr} + k'^0 \exp\left[-\left(\frac{\Delta E'}{RT}\right)\right] \qquad (1)$$

where τ (*T*) is the lifetime of the probe at *T* K. Table 3 shows the results of the thermodynamic analysis. When using $[Ru(phen)_3]^{2+}$ as a probe, the order of $\Delta E'$ (α-helix < silk I < silk II) agreed with that of the emission peak wave number (α-helix > silk I > silk II) suggesting that the MLCT energy level decreases in the order, α-helix > silk I > silk II. However, the order of $\Delta E'$ for the $[Ru(bpy)_3]^{2+}$ in the silks did not coincide with that of the emission peak wave number. This might be due to the change in the shape of the energy function, which could be caused by a strong interaction between the silk II and $[Ru(bpy)_3]^{2+}$ due to the smaller size of the bipyridine complex than the phenanthroline one. Another reason for the result could be the increase of the ground state energy level, but a satisfactory interpretation for this is not possible.

4. Conclusion

Emission maximum wave number of the $[Ru(bpy)_3]^{2+}$ or $[Ru(phen)_3]^{2+}$ photoluminescent probe incorporated in silk shifted to high energy relative to that in water, suggesting that the polarity of the microenvironment in the silk is in the order, α-helix < silk < silk II. The probes must exist in the rigid microenvironment that causes the *luminescence rigidochromism* as evidenced from the fact that the lifetime of probes is almost three times as long as that in water.

The order of $\Delta E'$ (α-helix < silk I < silk II) for the $[Ru(phen)_3]^{2+}$ is in accordance with that of the emission peak wave number, suggesting that the MLCT energy level decreases in the order, α-helix > silk I > silk II. On the other hand, the order of $\Delta E'$ for the $[Ru(bpy)_3]^{2+}$ in the silks did not agree with that of the emission peak wave number, suggesting a probable strong interaction between $[Ru(bpy)_3]^{2+}$ and silk II.

Acknowledgements

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