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Mark GAIDUK, Vil GRIGORYANTS*, Andrei MIRONOV**, and Valentina RUMYANTSEVA***

SPECTRO-LUMINESCENT CHARACTERISTICS AND KINETICS OF Yb^{3+} COMPLEXES OF PORPHYRINS

(Presented by K. K. Rebane)

Results of spectro-luminescent investigations and measurements of luminescence kinetics of a number of ytterbium complexes of porphyrins which present interest as the IR region fluorescent markers of malignant tumors are presented. The Stark structure of Yb^{3+} levels is interpreted in the framework of a crystal field (CF) model via comparison with CFs of garnets. A comparative study is made of the efficiency of fluorescence excitation of Yb^{3+} and molecular portion of porphyrin complexes in liposomes and aqueous solutions (H_2O and D_2O). The effects observed are associated with both possible monomerization of complexes in liposomes and processes of radiationless relaxation of energy. The lifetime of the metastable level of Yb^{3+} in porphyrin complexes is found to be dependent on their aggregate states and on the number of Br atoms in bromo-substituted porphyrins. The complex Yb-2,4-di(α-methoxyethyl) DPIX was selected as the most perspective of fluorescent markers. For it the luminescence contrast between the tumor and healthy tissue reached approximately 90.

Rare-earth ions attract ever increasing interest as spectroscopic probes in such a field of investigation as medicalbiology, in addition to probes common to these fields, such as, in particular, porphyrins, whose rigid ring of tetrapyrrolic macrocycle is used for luminescent probing of, e. g., nucleotides [1]. Such porphyrins as derivatives of hematoporphyrin (HPD) are of wide usage in luminescent diagnostics of malignant tumors, and as photosensitizers for photodynamic treatment of such tumors [2, 3].

In [4–9] we reported new possibilities opened by using «probe in the probe», i. e. ytterbium complexes of porphyrins in diagnostics of malignant tumors in the near IR spectral region.

The present paper describes results of spectro-luminescent investigations and measurements of luminescence kinetics for a number of solutions and solid-phase complexes of some ytterbium porphyrin complexes. The Stark structure of Yb^{3+} levels in this isostructural series has been identified. The data obtained can help in selecting the most promising complexes for luminescent diagnostics of tumors in experimental animals, using the method of fiber-laser spectrofluorimetry.

Experimental

The block diagram of the fiber-laser spectrofluorimeter (FLSF) [4, 9] built around a two-fiber introscope (TFIS) used in biological experiments is given in [4, 9]. In spectro-luminescent studies of samples in the form of powders and solution, the conventional experimental arrangement (without TFIS) was used. As light sources, an LGN-70 He-Cd laser (λ=442 nm), LTI-702 or LTN-401 YAG-Nd lasers with oscillation frequency doubling (λ=532 nm) (for stationary luminescence measurements) and an LTI PCh-5 laser (for luminescence kinetics measurement)

* Institute of Radio Engineering and Electronics of the USSR Academy of Sciences. 141 120 Fryazino, Moscow region, akad. Vvedenskogo Sq. 1. RSFSR.
** Lomonosov Moscow Institute of Fine Chemical Technology. 117 531 Moscow, Vernadskogo Ave. 86. RSFSR.

were used. MDR-3 and MDR-4 served as monochromators. In luminescence kinetics measurements, an S1-91 oscillograph was used whose screen was photographed.

Results

The synthesis of Yb-complexes of porphyrins used in our experiments is described in [4, 7, 9]. Besides, two isomers of Yb-complex of tetrasulfophenylporphyrin (TSPP) (Yb-tetra(4-methoxy-2-sulphophenyl)tetraphenylporphyrin (No 1) and Yb-tetra(3-methoxy-4-sulphophenyl) TPP (No 2)) and Br-substituted porphyrins Yb-4Br-TPP and Yb-12Br-TPP were synthesized. Apart from solutions and aqueous liposome suspensions, we also studied solid-phase compounds of a number of Yb-porphyrins (with partially extinguished molecular luminescence) preserving ability for intracenter 4f-4f fluorescence of the Yb^{3+} ion. This constitutes an additional advantage of the lantanoid «probe in the probes» like Yb-porphyrins. As an example of such solid-phase fluorescence, Fig. 1 shows spectra of an Yb^{3+} complex with TSPP which, as seen from the Figure, preserve full similarity to the solid and liquid phases. The similarity mentioned proves the stability of the coordination sphere of the metal ion. Note that the existence of an isostructural series of lantanoid complexes is itself a substantial help in interpreting their electronic spectra, e. g. as based on the theory of the CF [10].

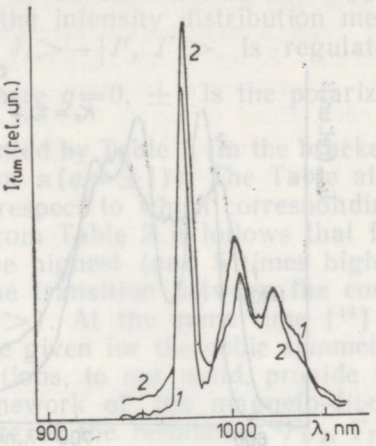

Fig. 1. Fluorescence spectra of powder (*1*) and 1% $NaHCO_3$ (*2*) solution of Yb-TSPP complex at 77 K.

The results of identifying the Stark structure of Yb^{3+} levels in some of the complexes investigated are shown in Fig. 2 (*1—5*).

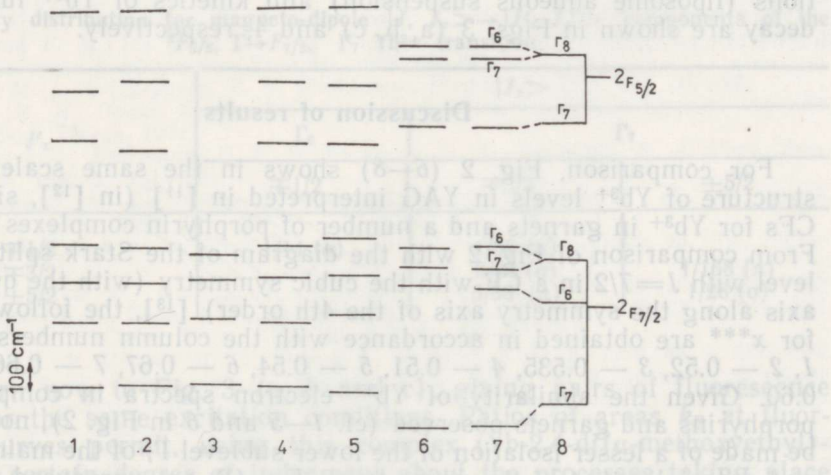

Fig. 2. The Stark structure of Yb^{3+} levels in Yb-2,4-di(α-methoxiethyl)DPIX complexes in phosphatidylcholin liposomes (*1*), 1% aqueous solution (*2*), in the form of powder at 77 K (*3*), in Yb-TSPP complex in liposomes (*4*), in 1% aqueous solution of soda (*5*); Yb^{3+} levels in YAG [11]: experimental values for rhombic (D_2) distortion of the cubic symmetry of the crystal (*7*) and calculated values for cubic symmetry only (*8*).

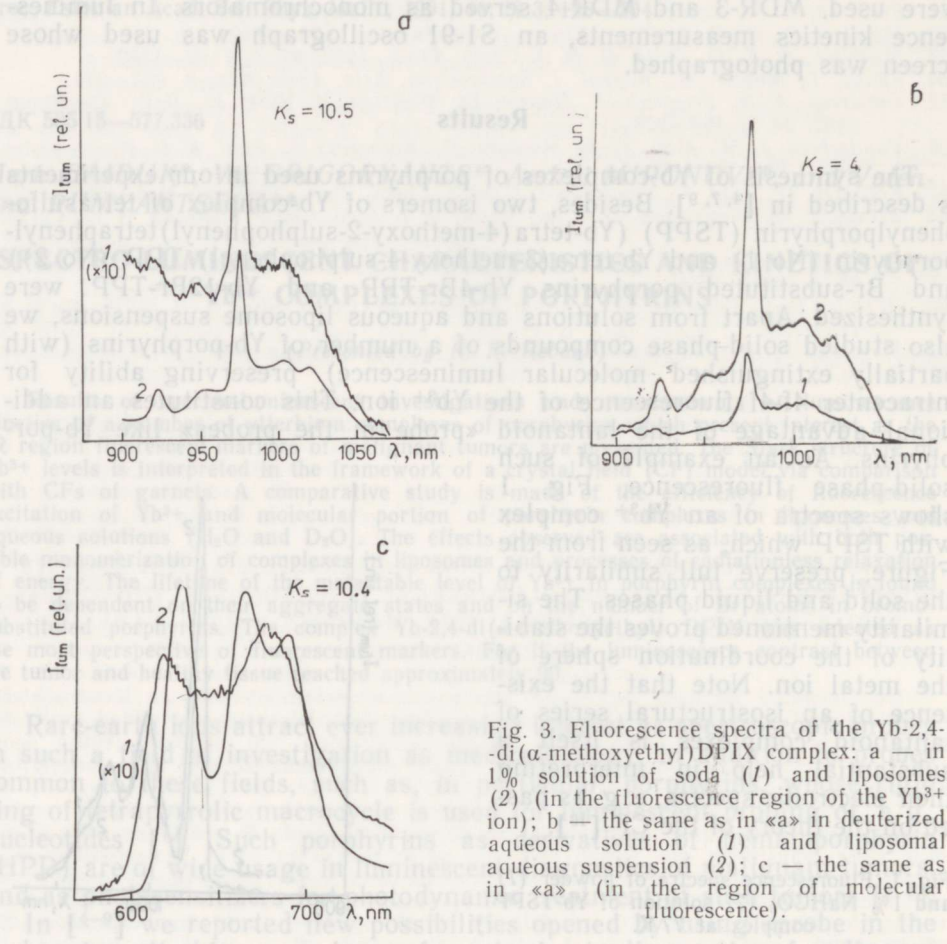

Fig. 3. Fluorescence spectra of the Yb-2,4-di(α-methoxyethyl)DPIX complex: a — in 1% solution of soda (*1*) and liposomes (*2*) (in the fluorescence region of the Yb^{3+} ion); b — the same as in «a» in deuterized aqueous solution (*1*) and liposomal aqueous suspension (*2*); c — the same as in «a» (in the region of molecular fluorescence).

The effect of inclusion of one of the Yb-porphyrin complexes into liposomes, which we observed together with deuteration of aqueous solutions (liposome aqueous suspension) and kinetics of Yb^{3+} luminescence decay are shown in Figs. 3 (a, b, c) and 4, respectively.

Discussion of results

For comparison, Fig. 2 (*6—8*) shows in the same scale the Stark structure of Yb^{3+} levels in YAG interpreted in [11] (in [12], similarity of CFs for Yb^{3+} in garnets and a number of porphyrin complexes is shown). From comparison of Fig. 2 with the diagram of the Stark splitting of the level with $J=7/2$ in a CF with the cubic symmetry (with the quantization axis along the symmetry axis of the 4th order) [13], the following values for x*** are obtained in accordance with the column numbers in Fig. 2: *1*, *2* — 0.52, *3* — 0.535, *4* — 0.51, *5* — 0.54, *6* — 0.67, *7* — 0.66, and *8* — 0.60. Given the similarity of Yb^{3+} electron spectra in complexes with porphyrins and garnets observed (cf. *1—5* and *6* in Fig. 2), notice should be made of a lesser isolation of the lower sublevel Γ_7 of the main level $^2F_{7/2}$ and especially that of the upper level $^2F_{5/2}$ (Γ_i are unreduced representations presented in the right-hand side of Fig. 2 [11]). The accompanying

*** According to [13], the parameter $x(-1 \leqslant x \leqslant 1)$ characterizes the relative contribution of the 6th- and 4th-order parameters into CFs, i.e. B_6^m/B_4^m (for $J \geqslant 3$).

decrease in the x values for Yb complexes as compared to Yb-YAG demonstrates a correspondingly larger contribution of the 6th-order CF parameters into metal-porphyrin CFs. Keeping in mind the aforesaid, the significant $^2F_{5/2}$ level splitting observed in Yb-porphyrin spectra is evidently due to a larger contribution of rhombic rather than tetragonal B_n^2 parameters ($n \leqslant 4$). This agrees with the concept of the rare-earth porphyrin complexes [14] and possible contribution into the CF made by two anion radicals of the Yb^{3+} ion (see also [15]).

Because of paucity of the structure of Yb^{3+} energy levels its detailed analysis is impossible without a resort to additional data. Of certain help may be an analysis of distribution of intensities of transitions between Stark components of the $^2F_{5/2}$, $^2F_{7/2}$ levels. The most characteristic feature of this distribution is a noticeable predominance (which was noted in [12]) of the intensity of the resonance $^2F_{5/2}$, $\Gamma_7 \rightarrow {}^2F_{7/2}$, Γ_7 transition, which may mean a not too large admixion of the low-symmetry component to the CF (an extremely small deviation of basis-wave functions of Yb^{3+} ($4f^{13}$) from «cubicity» was noted in [11]). If we assume that the $^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition is purely magneto-dipole, the intensity distribution mentioned above, which for transitions $|J, J_z> \rightarrow |J', J'_z>$ is regulated by the Wigner 3j-symbol $\begin{pmatrix} -J & 1 & J' \\ -J_z & q & J'_z \end{pmatrix}$ (here $q=0, \pm 1$ is the polarization, cf. [16] and also [10]), may be represented by Table 1 (in the brackets polarization type is given to be $\sigma(q=0)$ or $\pi(q=\pm 1)$). The Table also shows unreduced representations Γ_i with respect to which corresponding combinations $|J, J_z>$ are transformed. From Table 2 it follows that for $\Gamma_7 \rightarrow \Gamma_7$ transition of the probability will be highest (and 5 times higher than that for the $\Gamma_7 \rightarrow \Gamma_6$ transition for the transition between the components $\Gamma_7(|5/2, \mp 3/2>)$, $\Gamma_7(|7/2, \pm 5/2>)$. At the same time [13] it may be seen that the same components are given for the cubic symmetry with the maximum weight. These observations, to our mind, provide an exhaustive explanation (within the framework of the magneto-dipole nature of the transition) of the prevalence of the resonance $^2F_{5/2}$, $\Gamma_7 \rightarrow {}^2F_{7/2}$, Γ_7 transition of the Yb^{3+}, and serve as an indirect proof of a significant contribution of the cubic symmetry into the CF.

Table 1

The intensity distribution for magneto-dipole $|J, J_z> \rightarrow |J', J_z'>$ components of the $^2F_{5/2}$, $\Gamma \rightarrow F_{7/2}$, Γ_7 Yb^{3+} transition

	J'_z	$\lvert J_z>$		
		Γ_6	Γ_7	
		$\mp 1/2$	$\mp 3/2$	$\pm 5/2$
Γ_6	$\mp 1/2$	1/14 (σ)	1/56 (π)	
Γ_7	$\mp 3/2$		5/84 (σ)	1/168 (π)
Γ_7	$\pm 5/2$		5/56 (π)	1/28 (σ)

We turn now to Fig. 3 (a, b and c), giving pairs of fluorescence spectra for the same excitation conditions. Ratios of areas k_S at fluorescence curves permit, using this complex (Yb-2,4-di(α-methoxyethyl)-DPIX), a certain degree of judgement about the processes taking place when metal-porphyrin molecules penetrate into phospholipide vesicles. Constant values of k_S in Fig. 3 (a and b) evidently indicate that the effect of the liposomal membranes on the efficiency of Yb^{3+} fluorescence excitation is indirect via the molecular portion of the complex (this is

indirectly confirmed by a conclusion about a high efficiency of excitation energy transfer from porphyrin molecules to the metastable Yb^{3+} level drawn in [17]). At the same time, deuteration of water lowers the k_S values down to 4 (Fig. 3b), from which it follows that the effect observed is difficult to explain as caused by the monomerization of complexes during their penetration into liposomes as it is assumed in [18] for metal-free porphyrins. Otherwise, substituting ordinary water by heavy water in solutions and liposomal aqueous suspensions should not decrease k_S values so much (more than two and a half times). Evidently the penetration of complexes into phosphatidylcholine liposomes involves both polymerization (if the molecules tend to aggregate in aqueous solutions) and weaking of radiationless energy relaxation of the metal ion. Note that for Yb-TSPP complex the k_S values for ordinary and heavy water were 4 and 1.5, respectively, which probably means a lower aggregation capability of this complex as compared to the former, with radiationless relaxation by water molecules being of the same order of magnitude for the two complexes.

While examining the averaged values of τ given in Table 2, we noticed that they were greater for solid-phase Yb^{3+} complexes than for their solutions. Since in the case of solutions we also observed, as a rule, lower Yb^{3+} fluorescence intensity, these facts might be related to the extinguished effect of water molecules. The same correlation was observed for Yb-TSPP isomers No 1 and No 2. This makes the latter preferable as a fluorescence marker in the subsequent biological experiments.

Table 2

The average values of τ for some Yb^{3+} porphyrin complexes (μs)

Sample	Powder	Solution	Chloroform	Liposomes
Yb-TSPP	1.1	0.6	—	—
Yb-TSPP isomers				
N 1	0.9	0.5	—	—
N 2	1.0	0.7	—	—
Yb-TPP	0.6	—	—	—
Yb-4Br-TPP	0.8	—	—	—
Yb-12Br-TPP	0.9	—	—	—
Yb-2,4-di(α-methoxiethyl) DPIX	1.9	—	1.8	1.5

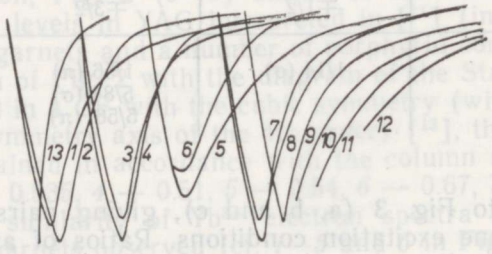

Fig. 4. Luminescence kinetics of Yb-porphyrin complexes (0.5 s/div.): Yb-TSPP (powder) (*1*); the same in 1% soda solution (*2*); isomer Yb-TSPP No 1 (powder) (*3*); the same in 1% soda solution (*4*); isomer Yb-TSPP No 2 (powder) (*5*), the same in 1% soda solution (*6*). Solid-phase complexes: Yb-TPP (*7*); Yb-4Br-TPP (*8*); Yb-12Br-TPP (*9*). Yb-2,4-di(α-methoxyethyl)DPIX complexes: powder (*10*); liposomal form (*11*) and chloroform solution (*12*). *13* — the pumping pulse.

It is interesting to note that an increase in τ accompanying transition from Yb-TPP complexes to 4- and 12-Br substituted porphyrins (see Fig. 4 and Table 2) correlates with the increase of photostability of the complexes in this series, which we observed. This is particularly interesting in connection with data on increased photochemical activity in haloid-substituted porphyrins with the increase of the number and atomic weights of halogens in the porphyrin cycle obtained at the Lomonosov Moscow Institute of Fine Chemical Technology.

As a result of spectro-kinetic investigations of Yb-porphyrin complexes, the Yb-2,4-di(α-methoxyethyl)DPIX complex was selected as the most promising for use as a fluorescent marker. In the biological experiments carried out using a fiber-laser spectrofluorimeter, the luminescence contrast between the sarcoma tumor and the healthy tissue was about 90 [19].

Further spectroscopic investigations are required, including those using kinetic methods and helium temperatures for better understanding of the relation between the spectro-kinetic and structural parameters of synthesized Yb-porphyrin complexes. This will help, in particular, in the search for and selection of the most efficient markers and photosensitizers.

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Mark GAIDUK, Vil GRIGORJANTS, Andrei MIRONOV, Valentina RUMJANTSEVA

PORFÜRIINIDE Yb^{3+}-KOMPLEKSIDE LUMINESTSENTSI SPEKTRAALKARAKTERISTIKUD JA KINEETIKA

On esitatud luminestsentsi spektraalomaduste ja kineetika mõõtmiste tulemused mõnede porfüriinide üterbiumikomplekside puhul, mis pakuvad huvi kui pahaloomuliste kasvajate infrapunases spektripiirkonnas fluorestseeruvad markerid. Kasutades analoogiat granaatkristallidega on interpreteeritud Yb^{3+} nivoode Starki struktuur ning pakutud seletus resonantse ülemineku $^2F_{5/2}(\Gamma_7) \rightarrow {}^2F_{7/2}(\Gamma_7)$ intensiivsuse domineerimisele spektris. On käsitletud fluorestsentsi intensiivsuse kasvu, mis kaasneb üleminekuga Yb-komplekside H_2O- ja D_2O-lahustelt nende liposoomides esinevatele vormidele. On tuvastatud Yb^{3+} $^2F_{5/2}$-nivoo eluea sõltuvus uuritud komplekside agregaatolekutest, aga ka broomiaatomite arvust broomasendajatega porfüriinide korral.

Марк ГАЙДУК, Вил ГРИГОРЬЯНЦ, Андрей МИРОНОВ, Валентина РУМЯНЦЕВА

СПЕКТРАЛЬНО-ЛЮМИНЕСЦЕНТНЫЕ ХАРАКТЕРИСТИКИ И КИНЕТИКА ЛЮМИНЕСЦЕНЦИИ Yb^{3+}-КОМПЛЕКСОВ ПОРФИРИНОВ

Результаты спектрально-люминесцентных исследований и измерений кинетики люминесценции некоторых иттербиевых комплексов порфиринов представляют интерес в качестве флуоресцентных меток злокачественных новообразований в ИК-диапазоне, где отсутствует фоновая люминесценция эндогенных веществ биотканей. Интерпретация штарковской структуры уровней Yb^{3+} проведена в рамках модели кристаллического поля (КП) сопоставлением с КП гранатов. Сделан вывод о большом вкладе в изученных комплексах параметров КП 6-го порядка относительно параметров 4-го порядка, а также ромбических параметров. Преобладание интенсивности резонансного $^2F_{5/2}(\Gamma_7) \rightarrow {}^2F_{7/2}(\Gamma_7)$-перехода находит объяснение при сохранении, как и в гранатах, доминирующей кубической компоненты КП в предположении магнитно-дипольной природы перехода.

Проведено сравнительное изучение эффективности возбуждения флуоресценции Yb^{3+} и молекулярной части порфириновых комплексов в липосомах и водных растворах (H_2O и D_2O). Наблюдаемые эффекты соотнесены как с возможной мономеризацией комплексов в липосомах, так и с процессами безызлучательной релаксации энергии.

Наблюдается зависимость времен жизни метастабильного $^2F_{5/2}$-уровня Yb^{3+} в комплексах порфиринов от их агрегатных состояний, а также от числа атомов брома в бромзамещенных порфиринах. Рассмотрена корреляция между этими факторами и эффективностью возбуждения флуоресценции или ее фотостабильностью.

В результате спектрально-кинетических исследований Yb-комплексов порфиринов одним из самых перспективных в качестве флуоресцентных маркеров был отобран комплекс Yb-2,4-ди (α-метоксиэтил) — DPIX. В последующих биологических экспериментах, проведенных с использованием волоконно-лазерного спектрофлуориметра в МНИОИ им. П. А. Герцена, значение люминесцентного контраста опухоль — здоровая ткань достигало 90.