LUMINESCENT MANIFESTATION OF THE DNA — BERBERINE INTERACTION

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The complete understanding of the therapy mechanism action of drugs is rather difficult without studies of the interaction of these compounds with biological objects on the molecular level. In our work, the results of investigations of the DNA — berberine guest molecules in amitozine (plant origin — *Chelidonium majus L.* — a drug with the anticancer and immune modulation properties) are presented.

The absorption, fluorescence, and phosphorescence of berberine are studied in a water solution without and in the presence of DNA. The adding of DNA macromolecules to berberine solutions doesn't lead to significant changes of the fluorescence and absorption spectra, but the fluorescence intensity dramatically increases (by ∼60 times). In our opinion, this phenomenon is connected with the intercalation of berberine molecules into DNA macromolecules. According to our investigations, the triplet excitations in DNA are localized mainly on berberine molecules bound to DNA. It is found that the average value of the triplet excitation displacement reaches at least the length of the twenty bases of DNA.

The results obtained can be a key to explain the molecular mechanism of the drug action.

Introduction

Amitozine is a new anticancer drug of the plant origin that was created by O.I. Potopalsky and his team at the Institute of Molecular Biology and Genetics of Ukraine.

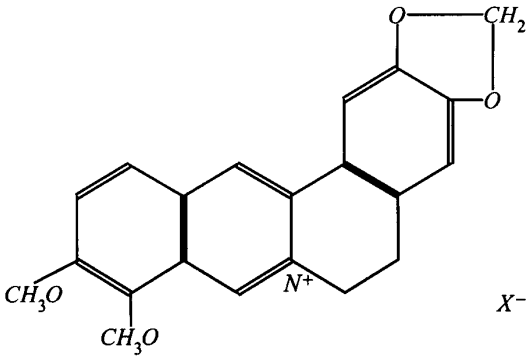

Fig. 1. Berberine molecule [2]

This drug has been already successfully tested in Ukraine. It was found that, in addition to the anticancer action, it has immunomodulating effect. Amitozine consists of several alkaloids. One of them is berberine [1] (Fig 1.). We established that the spectral properties of amitozine ride on the spectral properties of berberine. The present paper describes the spectral properties of a berberine molecule and the spectral manifestation of the DNA – berberine interaction

1. Experiment

Absorption spectra were recorded by a Specord UV VIS spectrophotometer. Luminescence and phosphorescence were studied by using a spectrometer of steady-state luminescence designed at our laboratory and a Cary Eclipse fluorometer. The software package Origin 6.1 was used to generate plots.

2.1. Spectral Properties of a Berberine Water Solution

The absorption, fluorescence, excitation spectra of phosphorescence and phosphorescence of a water solution were studied.

Absorption and fluorescence spectra are presented in Fig 2. The absorption spectra are situated in the region $\lambda < 500$ nm with the first long-wave maximum λ=440 nm. The fluorescence spectrum is displaced to the region 370—700 nm. Its blue edge overlaps with the red edge of the absorption spectrum, but the main fluorescence maximum is situated at λ=535 nm, i.e. rather far from the corresponding absorption spectrum maximum. So the Stoke's shift is significant. The latter could mean some transformation of a berberine molecule after the photon absorption takes place. At the same time, the fluorescence excitation spectra are close to the absorption berberine spectra, which proves the same origin of the absorption and emission species.

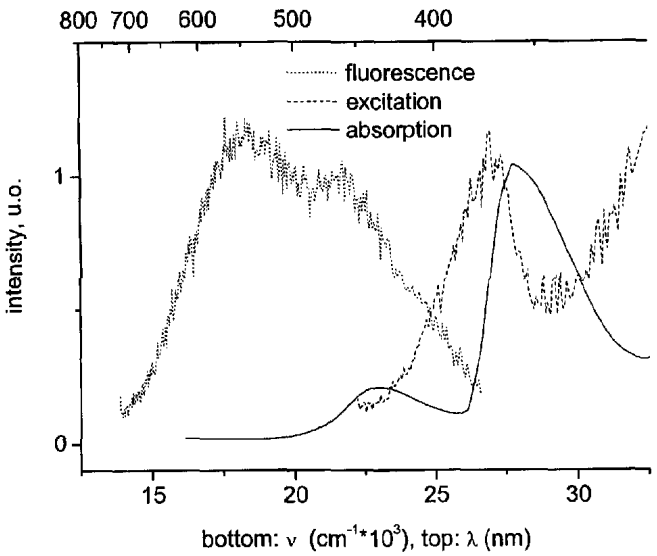

Fig. 2. Fluorescence, absorption and excitation spectra of a berberine water solution, λ_{exc}=366 nm, T = 293 K ($C = 5{\cdot}10^{-5}$ mole/l)

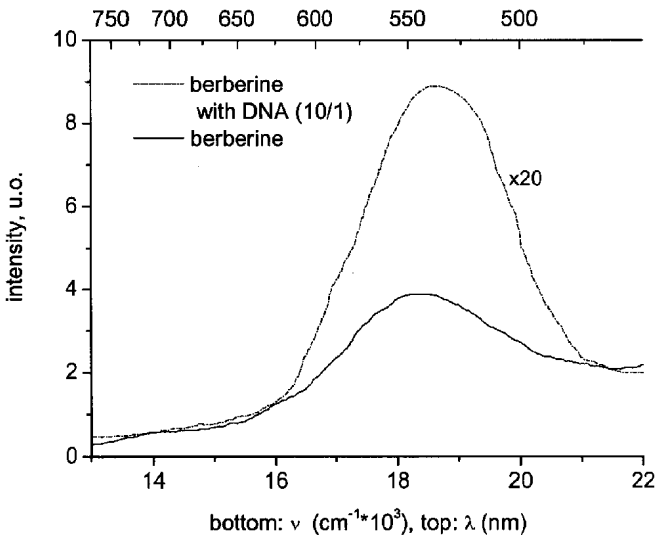

Fig. 3. Fluorescence of a berberine water solution, λ_{exc}=366 nm, T = 293 K ($C = 5{\cdot}10^{-5}$ mole/l)

2. Results and Discussion

2.2. Peculiarities of the Spectral Properties of a Berberine Water Solution in the Presence of DNA-Macromolecules

The adding of DNA macromolecules to water berberine solutions ($C = 5 \cdot 10^{-5}$ mole/l) starting from $C = 5 \cdot 10^{-4}$ mole/l (10 DNA bases per one berberine molecule) does not lead to significant spectral changes in the absorption and fluorescence spectra, although the intensity of berberine fluorescence rises dramatically (see Fig. 3).

The approximately 60-fold increase in the berberine fluorescence intensity was observed at the ratio of 1 berberine molecule to 10 DNA base points. Insignificant changes in the fluorescence and absorption spectra prove that there are no chemical binding of berberine molecules with DNA. The observed effect is connected, in our opinion, with reducing the number of freedom degrees (caused by the binding to DNA) of berberine molecules. For this reason, they can be deactivated without emission. The corresponding fluorescence yield enhancement has to be observed. It is known [3—7] that such a phenomenon takes place due to the physical binding of small molecules through the intercalation (the penetration into a macromolecule between DNA bases) or groove binding.

So, one of the amitozine drug components, namely a berberine molecule, possesses the ability to bind to a DNA-macromolecule.

2.3. Phosphorescence of Berberine. Triplet-Triplet Excitation Energy Transfer from DNA to Intercalated Berberine Molecules

Berberine doesn't manifest the phosphorescence at ambient temperatures. We observed the phosphorescence emission of a berberine water solutions at 77 K.

The berberine phosphorescence spectrum (Fig. 4) is observed in the region of 380—630 nm with the maximum at 510 nm. The berberine phosphorescence is not typical, because, as follows from Figs. 3 and 4, the phosphorescence spectrum of berberine is more short-wave than that of the berberine fluorescence. This is a very unusual case. The possible explanations are as follows:

— The observed phosphorescence is related to the radiative emission of the $T_2 \rightarrow S_o$ conversion,

— The phosphorescence appears if the second singlet level is excited. During the excitation life-time, the berberine π-electron system transforms into two nonconjugated chromophores. As a result, we observe the phosphorescence of these new chromophores.

The analysis of these two hypotheses leads to many other questions. The answers can be derived on the basis of additional investigations.

Studying the phosphorescence of the DNA and berberine binary water solution allows us to conclude that the triplet-triplet excitation energy transfer from

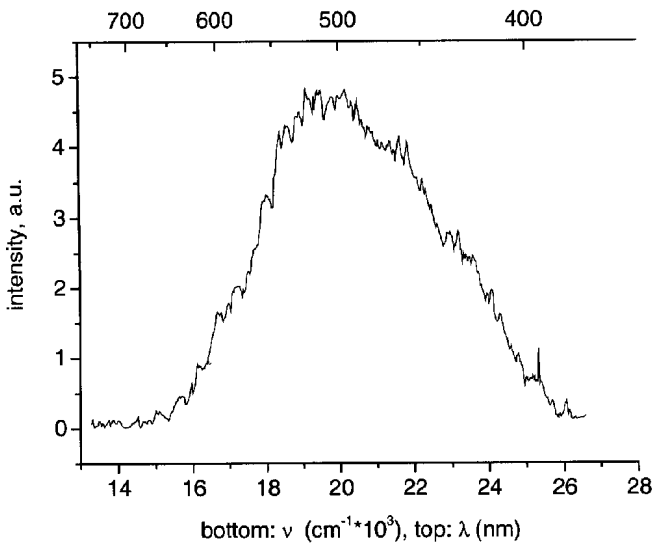

Fig. 4. Phosphorescence spectrum of a berberine water solution, $T = 77$ K

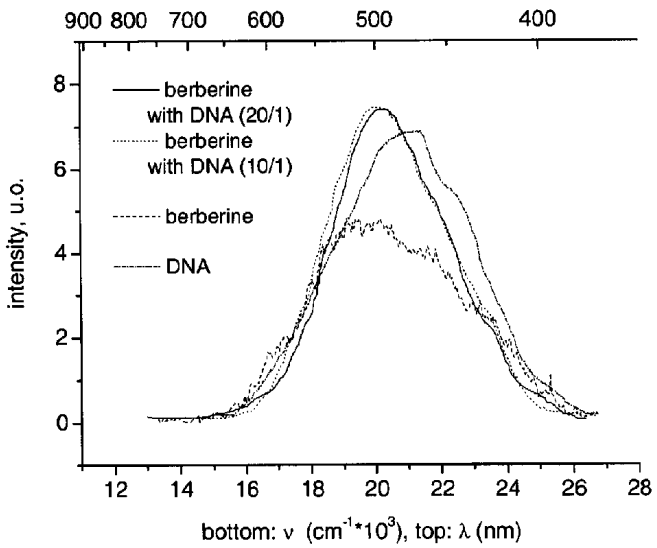

Fig. 5. Phosphorescence spectra of the DNA solutions, $T = 77$ K

DNA to berberine molecules takes place. We observed the DNA phosphorescence quenching by berberine molecules for values of the DNA base pairs/berberine molecules ratio from 10:1 to 20:1.

The data presented in Fig. 5 prove that the efficiency of quenching is not decreased significantly under the variation of the berberine — DNA base-pairs ratio from 1:10 to 1:20. It means that the triplet excitation in DNA can spread at least at the distance that corresponds to 20 DNA base pairs. Thus, the berberine molecules act as the traps for mobile triplet electronic excitations in DNA.

Conclusions

Berberine manifests the ability of the binding with DNA.

Molecules of berberine are the traps of triplet excitations in DNA.

The obtained results can be the key for understanding the molecular mechanism of the therapy effect of amitozine.

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ВПЛИВ ВЗАЄМОДІЇ ДНК—БЕРБЕРИН НА ЇХ ЛЮМІНЕСЦЕНЦІЮ

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Р е з ю м е

Повністю розуміти механізм терапевтичної дії ліків досить важко без вивчення взаємодії цих сполук з біологічними об'єктами на молекулярному рівні. Подано результати досліджень комплексу домішкових молекул ДНК—берберин в амітозині (рослинне походження — *Chelidonium majus L.* — ліки з антиканцерогенними та імуномодулюючими властивостями).

Досліджено поглинання, флуоресценцію і фосфоресценцію берберину у водному розчині за наявності ДНК і без неї. Додавання макромолекул ДНК до розчину берберину не викликає значних змін у спектрах флуоресценції та поглинання, але інтенсивність флуоресценції значно зростає (приблизно до 60 разів). На нашу думку, це явище пов'язане з інтеркаляцією молекул берберину у макромолекули ДНК. Згідно з нашими дослідженнями триплетні збудження в ДНК локалізовані головним чином на молекулах берберину, зв'язаних з ДНК. Визначено, що середня величина зміщення триплетного збудження сягає довжини щонайменше 20 ланок ДНК.

Отримані результати можуть бути важливими для пояснення молекулярного механізму дії ліків.