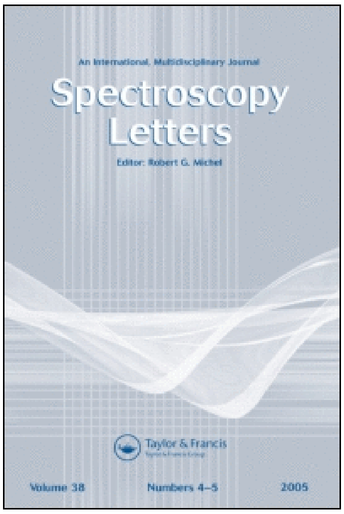

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FLUORESCENT PROPERTIES OF VITAMIN A DERIVATIVE, RETINYL PALMITATE, IN BINARY SOLVENTS WITH AQUEOUS PHASE

Key words: vitamin A, retinyl palmitate, fluorescence properties

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SUMMARY

Fluorescence emission and fluorescence excitation spactra of retinyl palmitate molecules in binary water/p-dioxane and water/isopropyl alcohol solvents under different solvents polarities were studied. The binary solvents polarities were changed by changing the molar ratios of water to p-dioxane (or isopropyl alcohol). It was suggested that the observed changes of spectral properties were caused by the formation of different retinyl palmitate self-organised structures that were induced by environments of different polarity. The role of non (low) polar components of binary solvents in the process of resultant structures creation is also discussed.

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INTRODUCTION

In solutions of different polarity and in binary solvents, vitamin A and vitamin A analogues (mostly with long hydrocarbon chains) exhibit characteristic interactions with the solvent [1]. Investigations of vitamin A analogues in different physical and chemical conditions have shown that those analogues exhibit specific properties in a binary solvent such as a mixture of ethyl alcohol and water [2]. It was suggested that the observed changes of spectral properties of investigated analogues cannot be explained by simple solvent effect [2-4] and cannot be related only to the different polarity of binary solvents used [5]. It was also suggested that the changes of spectral properties of retinyl palmitate (RP) analogue can be mostly induced by polar (water) component of binary solvent [5,6]. However, the results of retinyl palmitate fluorescence studies obtained in our previous investigations suggested that the mechanism of interaction between RP molecules in binary solvents cannot be explained only by the formation of retinyl specific structures forced by water [6]. Some evidences have been found that non (low) polar components of binary solvents could play the role in induction of specific RP structures. In the presented paper systematic investigations of the spectral properties of retinyl palmitate in binary water/p-dioxane and water/isopropyl alcohol solvents were undertaken. The polarities of binary solvents were changed by adding different amount of water to compounds used for PR molecules introduction. This caused that resultant binary solvents polarities were similar although non (low) polar components were different. The fluorescence emission and fluorescence excitation spectra were measured.

EXPERIMENTAL

Chemicals

Retinyl palmitate was purchased from Merck. p-dioxane (1,4-Dioxane) and 2-Propanol (Isopropyl alcohol) spectroscopic grade were from Polskie

Odczynniki Chemiczne. Bidestilled water was purified in Milki-Q Plus and Milki-R0 Plus (millipore).

Preparation of Solution

Binary polar/non (low) polar solvents consisting of water and p-dioxane or water and isopropyl alcohol at various ratios were used. RP was dissolved in p-dioxane or isopropyl alcohol to give a starting concentration of 3.97×10^{-3} M. Subsequently, 0.25 ml of RP was added to the binary solvents containing different water mole fraction X and mixed thoroughly. The final RP concentration was kept constant at 4.9×10^{-5} M for all binary solvents used. The binary solvents were saturated with argon just before RP was added. Immediately after preparation, samples were taken from each solution for absorption, fluorescence emission and fluorescence excitation spectra measurements.

Apparatus and Analytical Methods

Fluorescence measurements (emission and excitation spectra) were performed using a Perkin-Elmer LS50 luminescence spectrometer. The absorption spectra were registered with a Cary spectrophotometer (Varian, Australia). All experiments were carried out at room temperature. The data were analysed using computer programs: SPECTRA CALC (Galactic Ind. Corp.) and PRISM (GrahPad Software, Inc.).

RESULTS AND DISCUSSION

There were examined the fluorescence properties of RP in binary water/p-dioxane (or isopropyl alcohol) solvents at different water mole fraction (X). The different water content in binary solvents as well as the different p-dioxane and isopropyl alcohol dielectric properties caused the resultant solvents to be characterised by their dielectric parameters in wide region. Hence, obtained solvents can mimic different dielectric environments [5,7].

The fluorescence emission and fluorescence excitation spectra were measured for RP in binary solvents for $X < 0.7$ and $X \geq 0.9$ at constant RP concentration of 4.9×10^{-5} M (compare [5,7]). The wavelengths of fluorescence excitation, λ_{exc}, were chosen as to emphasise the differences between RP fluorescence in different binary solvents. The results presented in this work are referred to fluorescence spectra obtained at $\lambda_{exc,1}=320$ nm, $\lambda_{exc,2}=370$ nm and for part of samples at $\lambda_{exc,3}=280$ nm. The similar role was applied to select the wavelengths of emission for excitation spectra registration. The excitation spectra were registered at $\lambda_{em,1}=480$ nm and $\lambda_{em,2}=570$ nm.

The Changes of RP Fluorescence Emission Spectra in Binary Water/p-dioxane and Water/ Isopropyl Alcohol Solvents

The behaviour of RP in binary solvents was monitored with fluorescence spectroscopy. Hence, appearing light emitting structures were named as fluorescence centres (Cs). The kind of fluorescence centres was dependent on the water content on the sample. By proper choice of wavelength of excitation the characteristic changes of fluorescence spectra relevant to binary solvents with different centres (CsI, CsII or CsIII) contribution could be displayed [5-7]. Fig. 1 presents several characteristic fluorescence spectra for RP in binary water/p-dioxane solvents at different water mole fraction X. Curves 1-4 (left axis) were obtained at excitation of $\lambda_{exc}=320$ nm. Curves 5, 6 (right axis) were registered at excitations of $\lambda_{exc}=370$ nm and curve 7 at excitation of $\lambda_{exc}=280$ nm. The curves 1-3 ($\lambda_{max}=490$ nm) represent the fluorescence spectra of RP in binary solvents $0 < X < 0.62$ at the excitation of $\lambda_{exc}=320$ nm. These spectra are characteristic of fluorescence centres CsI [5]. Instead, the curve 4 comprises contribution of both CsI and CsIII. Fluorescence centres CsIII are created in binary solvents for the highest water mole fractions and CsIII are in dynamic equilibrium with CsI [6,7].

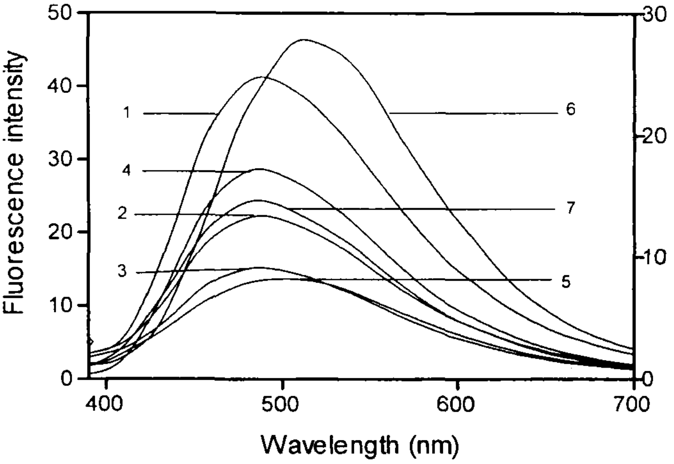

FIG. 1. Corrected fluorescence spectra of RP in binary water/p-dioxane solvent registered at different water mole fraction X: curve 1, X=0.0; curve 2, X=0.35; curve 3, X=0.62; curve 4, X=0.99, excitation at 320 nm (left axis) and curve 5, X=0.35; curve 6, X=0.62, excitation at 370 nm; curve 7, X=0.99, excitation at 280 nm (right axis).

In the case of longer wavelength of excitation (λ_{exc}=370 nm), the fluorescence spectra are batochromically shifted with increasing X value (Fig. 1, curves 5 and 6). The maximal spectral shift and the maximal fluorescence intensity were observed for RP in binary water/p-dioxane solvent for X=0.62. This is probably caused by optimal conditions for CsII, which are created due to dielectric enrichment [5]. Subsequent X increase, for X > 0.9 causes shorter wavelength shift of fluorescence spectra, which in turn indicates the presence off CsIII. Typical short wavelength fluorescence spectrum is obtained at λ_{exc}=280 nm (Fig. 1, curve 7). This spectrum is mostly generated by CsIII, which are in equilibrium with CsI (see also curve 4).

Fig. 2 demonstrates characteristic fluorescence spectra of RP in binary water/isopropyl alcohol solvents. Similarly to previous solvents, the RP

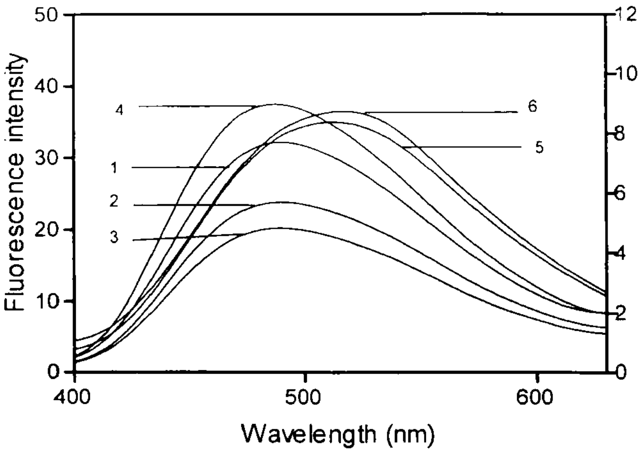

FIG. 2. Corrected fluorescence spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fraction X: curve 1, X=0.0; curve 2, X=0.30; curve 3, X=0.50; curve 4, X=0.94, excitation at 320 nm (left axis) and curve 5, X=0.30; curve 6, X=0.50, excitation at 370 nm (right axis).

fluorescence spectrum (λ_{exc}=320 nm) for 0 < X < 0.5 may by ascribed to CsI which RP creates in these solvents (curves 1-3, λ_{max}=490 nm). Instead, fluorescence spectra of RP in solvents with X > 0.9 are shifted towards shorter wavelengths (Fig. 2, curve 4). It is probable that there are contributions of two kind of fluorescence centres: CsI and CsIII which are created in water/isopropyl alcohol solvents with high water mole fraction. Analytically subtracted CsIII fluorescence spectrum is shifted towards shorter wavelength with respect to that one of CsI (Table 1, and [6]).

The fluorescence spectra obtained at longer wavelength excitation (λ_{exc}=370 nm) are shifted towards longer wavelengths and the fluorescence intensity is changed with increasing X (Fig.2, curves 5 and 6). The biggest spectral shift and the highest fluorescence intensity were observed for binary water/isopropyl

TABLE 1

The maxima of fluorescence emission spectra of fluorescence centres (in nm)

solvent	kind of centre		
	CsI	CsII	CsIII
water/p-dioxane	490	514	490
water/isopropyl alcohol	490	530	469

alcohol solvent with X=0.50. It is reasonable to assume that again CsII (in equilibrium with CsI) are mostly responsible for these spectral characteristics. It was found that RP creates CsII in binary water/isopropyl alcohol solvent for very narrow X region, close to X=0.50. It is assumed that CsII may be created due to non-specific solvation leading to dielectric enrichment. It is important to emphasise that optimal conditions for CsII creation in binary solvents with p-dioxane and isopropyl alcohol are a bit different: for $X \cong 0.62$ for p-dioxane and $X \cong 0.50$ for isopropyl alcohol. This result suggests that apart from earlier observation [6,7], both p-dioxane and isopropyl alcohol may induce different structures of fluorescence centres in binary solvents due to different dielectric properties.

Table 1 presents wavelengths corresponding to maxima of CsI, CsII and CsIII fluorescence spectra. The CsI fluorescence spectra are independent on solvent used. However, the maxima of fluorescence spectra of CsII and CsIII are influenced by solvent used for RP introduction into binary solvents.

The Changes of Fluorescence Excitation Spectra of RP in Binary Water/p-dioxane and Water/Isopropyl Alcohol Solvents with Different Water Mole Fraction X.

It was expected that the presence of different RP fluorescence centres in binary solvents should cause changes in fluorescence excitation spectra. Different wavelengths of irradiation (excitation, λ_{exc}) cause that different fluorescence centres emit light with different efficiency. This phenomenon implies the character of excitation spectrum, i.e. $I_{em} = f(\lambda_{exc})$.

Fig. 3 shows several characteristic fluorescence excitation spectra of RP in binary water/p-dioxane with different water mole fraction X for two wavelengths of observation. The wavelengths λ_{em} = 480 nm is from the narrow region close to the maximum of fluorescence band of CsI and λ_{em} = 570 nm corresponds with the longer part of fluorescence spectrum of CsII [5]. The curve 1 (left axis) and curve 4 (right axis) on Fig. 3 represent fluorescence excitation spectra of RP in pure p-dioxane and are characteristic of CsI. However, the excitation spectra of binary solvents containing CsII exhibit specific character. Three distinct maxima in the excitation spectra can be clearly observed. This interesting shape of the excitation spectrum is observed in the binary solvent with X = 0.62 and very close to this value. So, this phenomenon is observed for solvents containing CsII for two chosen wavelength of registration: 480 nm (Fig. 3, curve 2, left axis) and 570 nm (Fig. 3, curve 5, right axis). This may be connected with the fact that CsII exist in dynamic equilibrium with CsI and their fluorescence spectra can partly overlap [6]. As a result, the characteristic regions of CsII excitation are clearly seen in excitation spectrum registered at both 480 nm and 570 nm.

Fig. 3, curve 3 (left axis) shows an excitation spectrum of RP with high water mole fraction (X > 0.9). New region of wavelengths exciting fluorescence can be distinguish in these excitation spectra. In addition to maximum of excitation characteristic of CsI (Fig. 3, curve 1), new short wavelength region of excitation (about 280 nm) appeared. As it was discussed before, the excitation

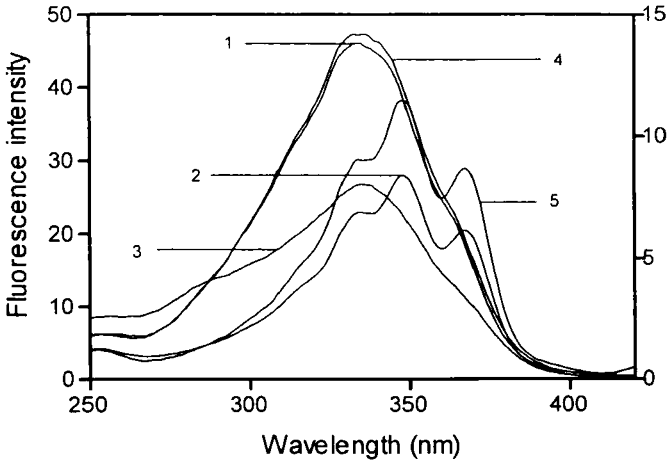

FIG. 3. Fluorescence excitation spectra of RP in binary water/p-dioxane solvent registered at different water mole fraction X: curve 1, X=0.0; curve 2, X=0.62; curve 3, X=0.99, observation at 480 nm (left axis) and curve 4, X=0.0; curve 5, X=0.62, observation at 570 nm (right axis).

with λexc = 280 nm induced "new" emission, which can be ascribed to centres CsIII. These centres are created by RP in binary water/p-dioxane solvents with X > 0.9 [6].

Fig. 4 presents example of typical fluorescence excitation spectra for RP in binary water/ isopropyl alcohol solvents with different water mole fraction X. Curve 1 (left axis) and curve 4 (right axis) represent fluorescence excitation spectra of CsI in pure isopropyl alcohol. Curve 2 (left axis) and curves 5 and 6 (right axis) with marked vibronic structure in the shape of sketch of bands, are characteristic for excitation spectra of CsII, that may be created by RP in binary water/isopropyl alcohol.

Similarly to fluorescence emission spectra of solutions with X > 0.9, specific shape of fluorescence excitation spectra in this X region can by found. Fig. 4, curve 3 demonstrates the fluorescence excitation spectrum of RP in solution with

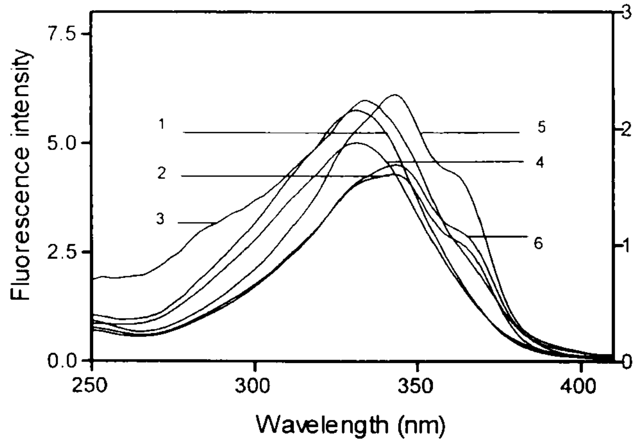

FIG. 4. Fluorescence excitation spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fraction X: curve 1, X=0.0; curve 2, X=0.60; curve 3, X=0.96, observation at 480 nm (left axis) and curve 4, X=0.0; curve 5, X=0.50; curve 6, X=0.60, observation at 570 nm (right axis).

X = 0.96. Again, in addition to longer wavelength band centred at 330 nm, new short wavelength region of excitations (centred at about 280 nm) was registered. It is plausible to assume that these changes are caused by CsIII, which are created in binary water/isopropyl alcohol with similar mechanism that led to creation of CsIII in binary water/p-dioxane solvents.

Obtained results indicate that fluorescence properties of RP in binary solvents change and are dependent on value of water mole fraction X. This is reflected by the fact that retinol palmitate can create certain, self-organised structures due to selective solvation of retinoid molecules by molecules of the binary solvent. These molecular structures can consist of one or more RP molecules till systems like micelles or microcrystals making the solvent turbid. Hence, depending on water molar ratio (X) there were distinguished three regions of retinal palmitate characteristic behaviour: (I) 0≤ X ≤ 0.6, (II) 0.6< X< 0.9 and (III) X ≥ 0.9. In regions (I) and (III) there were found fluorecent molecular structures. Instead, in region (II) RP molecules take part in creation specific structures that make the solvent turbid.

It occurred that retinyl palmitate excited state is more reactive than its ground state. Therefore fluorescence study should provide us with precise information about retinyl palmitate behaviour after light excitation. We have applied this method to demonstrate that RP molecules cannot be treated as uniformly distributed, separated molecules. But they can create (depending on water content of the binary solvent) different self-organised structures. There were found three fluorescent centres, CsI, CsII and CsIII. The contribution of these centres to resultant fluorescence spectra was dependent on dielectric properties of binary solvents which were changed by changes of X. The polar or low polar components of binary solvent (p-dioxane and isopropyl alcohol) were chosen to be different in structure and dielectric properties. Potentially, these components may be itself "organised" and may create different structures with water molecules. That is why the different mechanisms of RP fluorescence centres creation were expected. Obtained data allowed to suggest similar mechanism for CsI, CsII and CsIII creation in both systems. However, there was observed some influence of apolar (p-dioxane) or low-polar (isopropyl alcohol) components used to incorporate RP into binary solvents on RP fluorescence properties (compare Table 1).

This finding should be considered by those who use spectroscopic methods to investigate retinoids properties and use water (buffer) as a medium for these studies (compare [8] and literature therein).

REFERENCES

1. Horwitz, J., Heller, J. Properties of the Chromophore Binding Site of Retinol-binding Protein from Human Plasms. J. Biol. Chem. 1974, 249: 4712-4719.

2. Berni, R., Clerici, M., Malpeli, G., Cleris, L., Formelli, F. Retinoids: in vitro Interaction with Retinol-binding Protein and Influence on Plasma Retinol. FASEB J. 1993, 7: 1179-1184.

3. DeCoster, B., Christensen R. L., Gebhard, R., Lugtenburg, J., Farhoosh, R., Frank, H. A. Low-lying electronic State of Carotenoids. Biochim. Biophys. Acta, 1992, 1102: 107-114.

4. Li, X. -Y., Liu, R. S. H. Photostationary State Compositions of Retinal and Related Compounds Included in β-Lactoglobulin. Effects of Protein Host on Isomer Distribution of Polyene Substrates. Photochem. Photobiol. 1995, 62: 361-366.

5. Drabent, R., Bryl, K., Smyk, B., Ulbrych, K. Retinyl Palmitate in Water Environment. J. Photochem. Photobiol. B: Biology, 1997, 37: 254-260.

6. Bryl, K., Drabent, R., Olszewska, T. Fluorescent Investigation of the Vitamin A Self-organisation in Binary Solvents. *Proceedings of 5th International Conference on Methods and Applications of Fluorescence Spectroscopy*, 1997, Berlin, Germany, p.19.

7. Drabent, R., Bryl, K., Olszewska, T. Water Environment Forces Retinyl Palmitate to Create Self-organised Structures in Binary Solvents. *submitted for publication in J. Fluorescence.*

8. Crouch, R. K., Chader, G. J., Wiggert, B., Pepperberg, Retinoids and the Visual Process, Photochem. Photobiol. 1996, 64: 613-621.

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