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A Water Environment Forces Retinyl Palmitate to Create Self-Organized Structures in Binary Solvents

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The interaction between retinyl palmitate molecules and their environment in binary water/*p*-dioxane and water/isopropyl alcohol solvents was studied under different solvent polarities. The binary solvent polarities were changed by changing the molar ratios of water to *p*-dioxane (or isopropyl alcohol). The fluorescence emission and fluorescence excitation spectra were analyzed. In binary solvents, the interaction between retinyl palmitate molecules and environments of different polarities leads to the self-organization of retinyl palmitate, and as a result, different fluorescence centers are created. The similar fluorescence properties of these centers in different binary solvents were interpreted as a water driving force inducing the self-organization of retinyl palmitate. The possible consequences of this phenomenon for misleading interpretation of model studies of retinol interaction with retinol transporting proteins are also discussed.

KEY WORDS: Retinyl palmitate; self-organization; fluorescence properties; binary solvents.

INTRODUCTION

Liver stellate cells store vitamin A, as retinyl esters (mainly retinyl palmitate) in lipid droplets located in their cytoplasm.[1-3] To fulfill the organism's needs, vitamin A stores are mobilized from the liver into the serum, where vitamin A circulates as retinol, bound to retinol binding protein (RBP). Such mobilization implies several steps (retinyl ester hydrolysis, retinol binding to RBP, secretion into the serum), which are still incompletely understood and sometimes controversial.[4,5] Irrespective of their cellular location, all RBPs share the ability to bind, transport, and release retinoids. The molecular mechanisms of these reactions have not been elucidated yet. In particular, the route of retiol transfer through and between membranes is unknown.[6-8]

Retinyl esters can be hydrolyzed in model systems (*in vitro*) by a large variety of enzymes, whose specificity and relevance in physiological conditions are difficult to assess. This is due mainly to the poor characterization of the water/lipid interface where hydrolysis occurs, because of the hydrophobicity of the substrate.[9] A similar problem is encountered when retinol binding to RBP or direct vitamin A transfer into the required places in the cell is investigated. RBP is a water-soluble species. However, the solubility of retinoids in water is very low.[10] Therefore, most experiments aiming at clarifying the structural features of retinoids that may be required for their interaction with binding proteins or for their direct transport in the cell have been performed in binary solvents. Concentrated alcoholic solutions of retinoids were added to water (buffer) or water (buffer) containing water-soluble compounds.[11-14] The poor characterization of the water/vitamin A systems impedes the interpretation of experimental data and might be the cause of controversial results [15, and references therein].

In our previous studies[16] it was found that retinyl palmitate introduced into water via *p*-dioxane created a dispersion phase in which the effective concentration of vitamin was 16 times higher than the solubility-limited value. It was suggested that the water molecules them-

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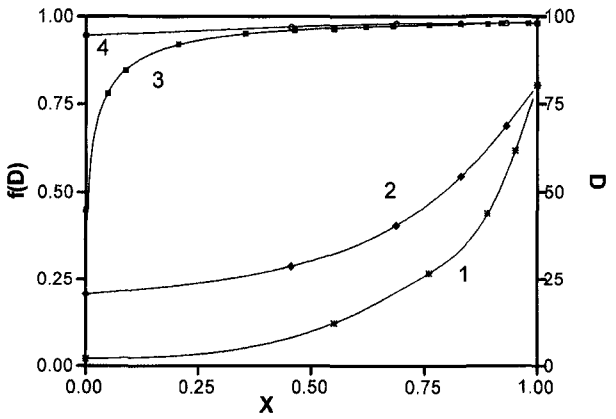

Fig. 1. Dielectric constant D of the binary solvents water/*p*-dioxane (1) and water/propyl alcohol (2) and dielectric polarity $f(D)$ of the binary solvents water/*p*-dioxane (3) and water/isopropyl alcohol (4) as a function of the water mole fraction X.

selves can influence the interaction of retinoid molecules, leading to their self-organization. Such self-organized molecules could be transferred directly into the required places via the aqueous phase without any transporting protein in the cell. The specific solvent, water/other components of the cell (in biophysical language: the microenvironment, with changeable electric properties), can force the biological macromolecules to be in a "shape suitable for proper biological function."

The implications of previous studies might be deeper. If water strongly influences the solubility and structure of introduced water-insoluble retinyl palmitate molecules, it may be that the interaction of retinoids with the cell elements or with water-soluble species (e.g., RBP) should not be considered as an interaction in a well-defined, uniform solvent. To solve this problem we have undertaken a further, more systematic study of retinyl palmitate in binary solvents. The aim of the present investigation was to clarify whether (and, if yes, how) the water content of binary solvents can influence the properties of retinyl palmitate and can induce its self-organization in relation to the properties of different solvents, in which retinyl palmitate is well soluble: 1,4-dioxane and 2-propanol.

MATERIALS AND METHODS

Chemicals

Retinyl palmitate was purchased from Merck. *p*-dioxane (1,4-dioxane) and 2-propanol (isopropyl alcohol), spectroscopic grade, were from Polskie Odczynniki Chemiczne. Bidistilled water was purified in Milki-Q Plus and Milki-R0 Plus (Millipore).

Preparation of Solutions

Binary polar/nonpolar (or low-polarity) solvents consisting of water and isopropyl alcohol or water and *p*-dioxane at various ratios were used. This way, the number of water molecules per number of isopropyl alcohol or *p*-dioxane molecules varied. Hence, the number of water molecules per retinyl palmitate also varied. Such solvents exhibited different polarities $f(D)$ and dielectric constants, D (see Fig. 1). The resultant function $f(D)$, describing the property of the binary solvent, can be calculated as a function of the linear combination of respective dielectric constants D_m:[16,17]

$$D_m = x_1 D_1 + x_2 D_2 \quad (1)$$

$$f(D) = \frac{2(D_m - 1)}{2D_m + 1} \quad (2)$$

where x_1 and x_2 are the contributions of the mole fractions of polar and nonpolar components, respectively, and D_1 and D_2 are the respective dielectric constants of these components.

RP was dissolved in isopropyl alcohol (or *p*-dioxane) to give a starting concentration of 3.97×10^{-3} M. Subsequently, 0.25 ml of RP was added to the binary solvents and mixed thoroughly. The final RP concentration was kept constant at 4.9×10^{-5} M for solvents with isopropyl alcohol and *p*-dioxane. The binary solvents were saturated with argon just before RP was incorporated. Immediately after preparations, samples were taken from each solution for absorption, fluorescence emission, and fluorescence excitation spectrum measurements.

Analytical Methods

The methods of measurements and data analysis were described earlier in detail.[16] Briefly, the absorption spectra were measured with a Cary 3E spectrophotometer (Varian, Australia). Fluorescence measurements (emission and excitation spectra) were performed using a Perkin–Elmer LS50 spectrophotometer. All experiments were carried out at room temperature. The data were analyzed using computer programs: SPECTRA CALC (Galactic Ind. Corp.) and PRISM (GraphPad Software, INC.)

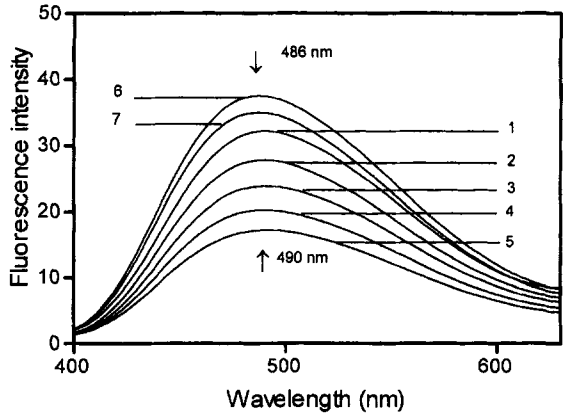

Fig. 2. Corrected fluorescence spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fractions X: curve 1, $X = 0$; curve 2, $X = 0.1$; curve 3, $X = 0.3$; curve 4, $X = 0.5$; curve 5, $X = 0.6$; curve 6, $X = 0.94$; curve 7, $X = 0.98$. Excitation at 320 nm. Arrows indicate the fluorescence maxima for curves 1–5 (490 nm) and for curves 6 and 7 (486 nm).

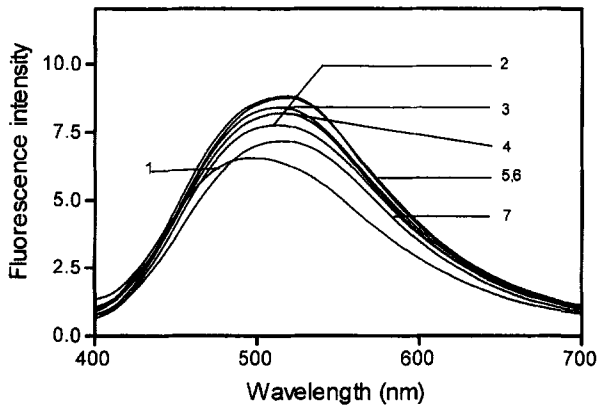

Fig. 3. Corrected fluorescence spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fractions X: curve 1, $X = 0$; curve 2, $X = 0.2$; curve 3, $X = 0.3$; curve 4, $X = 0.48$; curve 5, $X = 0.5$; curve 6, $X = 0.54$; curve 7, $X = 0.6$. Excitation at 370 nm.

RESULTS AND DISCUSSION

RP in binary water/isopropyl alcohol (or *p*-dioxane) solvents at different mole fractions (X) of the polar component (water) was studied. The absorption, fluorescence emission, and fluorescence excitation spectra were measured.

Fluorescence of Retinyl Palmitate in Binary Solvent Water/Isopropyl Alcohol

Fluorescence emission spectra of RP dissolved in pure isopropyl alcohol and in binary solvents at different mole fractions X of water were measured at two excitation wavelengths: $\lambda_{exc,1} = 320$ nm, close to $\lambda_{abs}^{max} = 326$ nm, and $\lambda_{exc,2} = 370$ nm, from the region of the long-wavelength region of the absorption spectrum. The same wavelengths were previously used to excite RP in water/*p*-dioxane solvent.[16]

The corrected RP fluorescence spectra, measured at $\lambda_{exc,1} = 320$ nm, are shown in Fig. 2. The fluorescence intensity decreases with increasing X (up to $X = 0.6$). In the region of $X \geq 0.9$, the fluorescence intensity increases and λ_{max} shifts toward shorter wavelengths. Instead, for excitation at 370 nm, the RP fluorescence spectra are shifted toward longer wavelengths, with a concomitant change in their intensity, different for different X values (Fig. 3). These results and the results obtained earlier[16] suggest that three groups of characteristic RP fluorescence should be distinguished, according to the water mole fraction in the binary solvent: (i) for $0 \leq X \leq 0.6$, (ii) for $0.6 < X < 0.9$, and (iii) for $X \geq 0.9$. The region of $0.6 < X < 0.9$ gives a turbid solution. The analysis of the intensity of light generated by the sample is complicated due to strong light scattering. That is why this region of X changes is not considered.

Fluorescence spectroscopy has an advantage over other techniques, because fluorescence is indicative of the chemical properties of the compounds, and spectroscopic measurements allow precise monitoring of the solute within an aqueous environment. In addition, the application of the proper wavelength of light for excitation facilitates the exposure of characteristic changes which take place in RP fluorescence in the first and third region of water mole fraction X in binary solvents.

Fluorescence of RP in Binary Solvent Water/Isopropyl Alcohol ($0 \leq X \leq 0.6$)

Figure 2 ($\lambda_{exc,1} = 320$ nm) demonstrates that the wavelength of the fluorescence maximum of RP in binary solvent water/isopropyl alcohol ($0 \leq X \leq 0.6$) does not change. This λ_{max}^{fl} in binary solvents equals $\lambda_{max}^{fl} = 490$ nm in pure isopropyl alcohol. Instead, the fluorescence intensity decreases with increasing X. Excitation of the sample with a longer wavelength ($\lambda_{exc,2} = 370$ nm) displayed a new spectral feature: fluorescence spectra shift toward longer wavelengths (Fig. 3). These effects suggest that RP in binary solvent water/isopropyl alcohol at $X \leq 0.6$ creates two types of fluorescence centers, CsI and CsII, which coexist in dynamic equilibrium.

The fluorescence of CsII appears when a longer wavelength of excitation is used. That is why these emis-

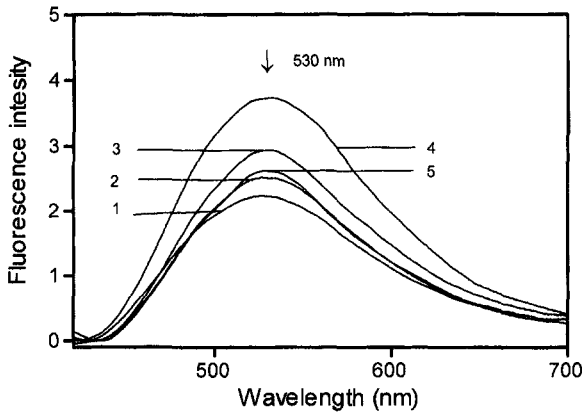

Fig. 4. Calculated fluorescence spectra of RP in binary water/isopropyl alcohol solvents: curve 1, $X = 0.3$; curve 2, $X = 0.48$; curve 3, $X = 0.5$; curve 4, $X = 0.52$; curve 5, $X = 0.54$. Calculations described in the text.

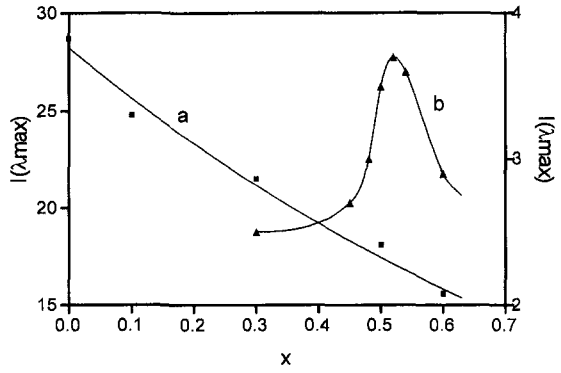

Fig. 5. Dependence of the maximum fluorescence intensity $I(\lambda^{max})$ on the water mole fraction X. Curve a, left axis, excitation at 320 nm; curve b, right axis. Explanation in text.

sion spectra should be considered as linear combinations of the fluorescence spectra of RP in pure isopropyl alcohol (CsI) and fluorescence spectra of CsII. Figure 4 presents the fluorescence spectra of CsII calculated by the method described earlier.[16] The maxima of calculated fluorescence spectra do not depend on X and λ^{fl}_{max} (CsII) = 530 nm. However, a strong dependence of fluorescence intensity on water content was observed. Figure 5 presents the change in average values of fluorescence intensity (I^{em}_{max}) for CsI (a) and for CsII (b) due to different water contents. Surprisingly, the change of the water mole fraction X in the binary solvent water/isopropyl alcohol ($0 \leq X \leq 0.6$) causes similar changes induced by water in water/*p*-dioxane (Fig. 6 in Ref. 16).

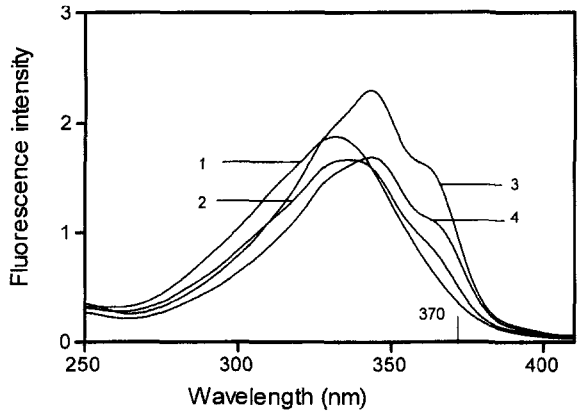

Fig. 6. Fluorescence excitation spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fractions X: curve 1, $X = 0.0$; curve 2, $X = 0.3$; curve 3, $X = 0.5$; curve 4, $X = 0.6$. Observation at 570 nm. The bar at 370 nm indicates the wavelength of excitation used in presented experiments.

The changes in the fluorescence properties of RP in binary solvents were supplemented by analysis of the excitation spectra, $I_{em} = f(\lambda_{exc})$. The excitation spectra were registered at two emission wavelengths: $\lambda_{em,1}$ = 480 nm and $\lambda_{em,2}$ = 570 nm. The first wavelength of emission is very close to the wavelength corresponding to the emission maximum of RP in pure isopropyl alcohol (λ^{fl}_{max} = 490 nm; Fig. 2). The $\lambda_{em,2}$ was chosen to be from the longer-wavelength part of CsII emission spectra. The excitation spectra of RP in binary solvents with different mole fractions of water, $X \leq 0.6$ at $\lambda_{em,2}$ = 570 nm, are presented in Fig. 6. A particularly interesting shape of the excitation spectra is observed in the binary solvents with $X \cong 0.5$–0.6. Three distinct maxima in those excitation spectra can be observed. Excitation and fluorescence spectra with similar structures are observed for RP in binary water/*p*-dioxane solvents[16] and for amphiphilic compounds in binary solvents.[18]

These results suggest that, irrespective of the different structures and properties of isopropyl alcohol and *p*-dioxane, the polar component of both binary solvents (water) induces similar self-organized RP structures. Spectroscopic similarities and regions of characteristic X values allow us to conclude that the structure of both fluorescence centers in these two binary solvents is similar. Centers of the first type (CsI) involve RP molecules surrounded mostly by isopropyl alcohol (or *p*-dioxane) molecules. Hence, CsII centers differ from CsI centers in terms of emission properties. Thus RP molecules in the electronically excited state (RP*) contribute to CsII. For more details see the Discussion in Ref. 16.

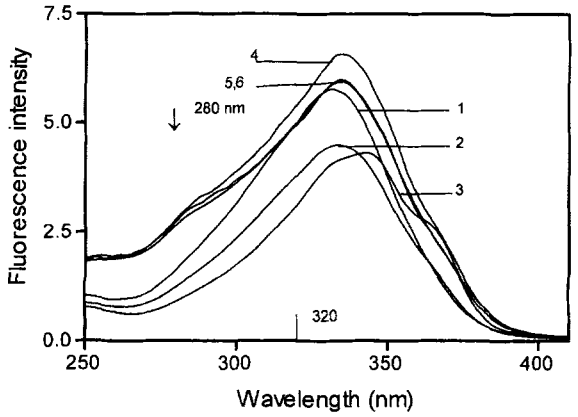

Fig. 7. Fluorescence excitation spectra of RP in binary water/isopropyl alcohol solvent registered at different water mole fractions X: curve 1, $X = 0.0$; curve 2, $X = 0.2$; curve 3, $X = 0.6$; curve 4, $X = 0.92$; curve 5, $X = 0.96$; curve 6, $X = 0.98$. Observation at 480 nm. The bar at 320 nm indicates the wavelength of excitation used in the presented experiments.

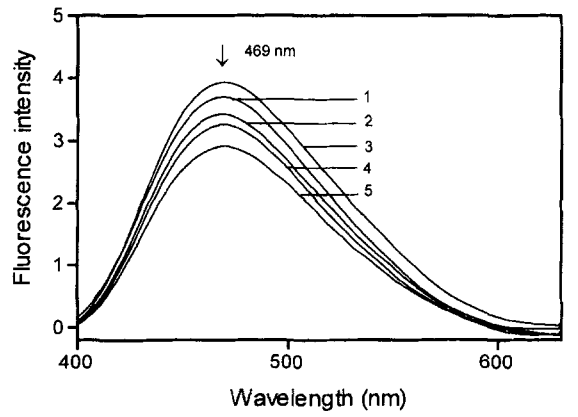

Fig. 8. Calculated fluorescence spectra of RP in binary water/isopropyl alcohol solvent: curve 1, $X = 0.90$; curve 2, $X = 0.94$; curve 3, $X = 0.96$; curve 4, $X = 0.98$; curve 5, $X = 0.99$. Calculations described in the text.

The question arises whether similar RP behavior can be observed in the most interesting X region, $X \geq 0.9$, for both binary solvents. Actually, this region defines the binary solvents for spectroscopic studies on vitamin A.

Fluorescence of RP in Binary Solvents, Water/Isopropyl Alcohol and Water/*p*-Dioxane, for a High Water Mole Fraction, $X > 0.9$.

Fluorescence emission spectra and fluorescence excitation spectra of RP in binary water/isopropyl alcohol and water/*p*-dioxane for $0.9 \leq X \leq 0.99$ were measured. In this X region, the dielectric constants of binary solvents are practically equal to the dielectric constant of water (Fig. 1).

Fluorescence of RP in Binary Water/Isopropyl Alcohol Solvents

The fluorescence spectra of RP in binary solvents shift toward shorter wavelengths with increasing X from 0.90 to 0.99 with respect to the fluorescence spectrum of RP in pure isopropyl alcohol (Fig. 2).

Figure 7 shows fluorescence excitation spectra (λ_{em} = 480 nm). The increase in X causes the appearance of a new band at a shorter-wavelength side of the excitation spectra (region of λ_{exc}, about 280 nm). This suggests that new (CsIII) fluorescence centers can be created. In connection with this, the fluorescence spectra obtained for $X \geq 0.9$ at λ_{exc} = 320 nm can be considered a linear combination of CsI and CSIII fluorescence spectra. Figure 8 shows the calculated fluorescence spectra of CsIII (λ_{inax} = 469 nm). The method of calculation is similar to that applied for CsII fluorescence spectra calculation.[16]

The contribution of CsIII fluorescence to the resultant fluorescence may be lowered. There are at least two reasons for this:

(a) the excitation is "at the longer-wavelength part" of a putative excitation band of CsIII (Fig. 7, curves 4–6); and

(b) simultaneously, this region is a region of intensive excitation of fluorescence centres CsI.

In order to compare the fluorescence of these three centers, the results of investigations of solvents with such water molar fractions and excited at wavelengths to expose the consecutive light emission by consecutive centers were considered. The fluorescence of CsI is the fluorescence of RP in pure isopropyl alcohol at λ_{exc} = 320 nm. CsII centers emit strong fluorescence when RP is placed in binary solvent water/isopropyl alcohol for $X \approx 0.5$. Their excitation is very effective at λ_{exc} = 370 nm (Figs. 3 and 5b). The registered fluorescence spectra were used for analytical subtraction of CsII fluorescence spectra (Fig. 4). The fluorescence of CsIII appears strongest for RP in binary solvents at $X \geq 0.9$ when short-wavelength excitation is applied (λ_{exc} = 320 nm). These spectra were used for calculation of the fluorescence spectra of CsIII (Fig. 8). The fluorescence spectra obtained from the experiments with such chosen parameters were normalized to the intensity maximum of each spectrum. Figure 9 presents calculated fluorescence spectra of CsI, CsII, and CsIII. The shifts of the CsII

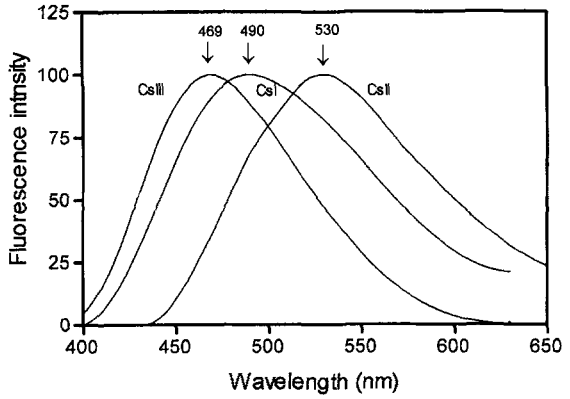

Fig. 9. Normalized fluorescence spectra of CsI, CsII, and CsIII for RP in binary water/isopropyl alcohol solvent.

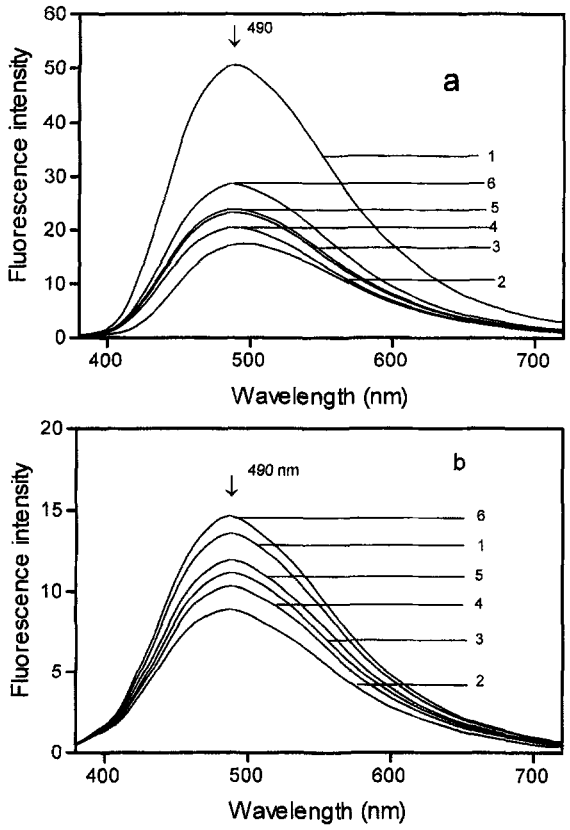

Fig. 10. Corrected fluorescence spectra of RP in binary water/*p*-dioxane solvent registered at different water mole fractions X: curve 1, $X = 0$; curve 2, $X = 0.94$; curve 3, $X = 0.96$; curve 4, $X = 0.97$; curve 5, $X = 0.98$; curve 6, $X = 0.99$; Excitation at 320 nm (a) and 280 nm (b).

and CsIII fluorescence spectra according to the fluorescence spectrum of CsI are as follows:

$$\Delta\lambda^{fl}_{max}\ (\text{CsII} - \text{CsI}) = 530\ \text{nm} - 490\ \text{nm} = 40\ \text{nm}$$

$$\Delta\lambda^{fl}_{max}\ (\text{CsI} - \text{CsIII}) = 490\ \text{nm} - 469\ \text{nm} = 21\ \text{nm}$$

*Fluorescence of RP in Binary Water/*p*-Dioxane Solvents*

The fluorescence of RP was measured in binary water/*p*-dioxane solvents for $X \geq 0.9$. Our preliminary data[16] demonstrated that these solvents were transparent. Hence, RP emission can be considered fluorescence of centers created by RP molecules. The wavelength of excitation was chosen to be $\lambda_{exc,1} = 320$ nm, $\lambda_{exc,2} = 370$ nm, and $\lambda_{exc,3} = 280$ nm. It was observed that RP molecules excited with $\lambda_{exc,2}$ for $X \geq 0.9$ emitted low-intensity fluorescence. The longer-wavelength parts of these bands were similar to the fluorescence spectra excited with $\lambda_{exc,1}$. This observation suggests that, if there are CsII centers in these solvents, their amount should be very low. Hence, for further investigations, excitations of $\lambda_{exc,1}$ and $\lambda_{exc,3}$ were chosen. Figures 10a and show the fluorescence spectra of RP for $X \geq 0.9$. The values describing registered spectra are listed in Table I. In order to compare the data for different excitations, the relative fluorescence intensity (I_r) versus the fluorescence intensity of RP in pure *p*-dioxane (I_0) was calculated. As can be seen from Fig. 10 and Table I, the fluorescence spectra registered for $X \geq 0.9$ are similar to the fluorescence spectrum of RP in *p*-dioxane. However, the value of I_r is different. RP excitation at $\lambda_{exc,3}$ is more effective than excitation at $\lambda_{exc,1}$ (Table I, columns 5 and 8). The fluorescence intensity of RP molecules excited at $\lambda_{exc,3}$ is higher than their fluorescence in *p*-dioxane (Table I, column 9, $I_r > 1$).

Figure 11 demonstrates the fluorescence excitation spectra of RP in binary water/*p*-dioxane solvent ($X > 0.9$) registered at $\lambda_{em} = 480$ nm. In order to separate the short-wavelength region of fluorescence excitation, the difference spectra were calculated. The excitation spectrum for RP in pure *p*-dioxane (an excitation spectrum of CsI) was subtracted from the excitation spectra registered at different X values. It was assumed that for $X > 0.9$, the light at wavelength of $\lambda_{exc} = 336$ nm excited mostly CsI. However, in the systems in which different light emitting forms and different created fluorescence spectra exist, these centers emit the light with different efficiencies and several phenomena may take place. For example, energy transfer may occur (reabsorption, reemission, resonance transfer) and the effect of the inner filter should be considered. The absorption bands of fluorescence centers overlap partially. It causes light that may be used for excitation of one kind of center to be

Table I. Fluorescence Parameters of RP in Dioxane and Water/Dioxane Solutions

		$\lambda_{exc,1}$ = 320 nm			$\lambda_{exc,3}$ = 280 nm			
No.	X	λ_{em}^{max} (nm)	I^1	$I_r = (I^1/I_0^1)$	λ_{em}^{max} (nm)	I^3	$I_r = (I^3/I_0^3)$	$I_r = (I_r^{280}/I_r^{320})$
1	2	3	4	5	6	7	8	9
1	0.00	490	I_0^1 = 50.5	1	490	I_0^3 = 14.1	1	1
2	0.94	488	17.5	0.35	487	8.9	0.63	1.82
3	0.96	489	23.3	0.46	489	11.2	0.79	1.72
4	0.97	489	20.4	0.40	489	10.3	0.73	1.83
5	0.98	488	24.0	0.47	489	11.9	0.84	1.79
6	0.99	488	29.3	0.58	488	14.6	1.04	1.79

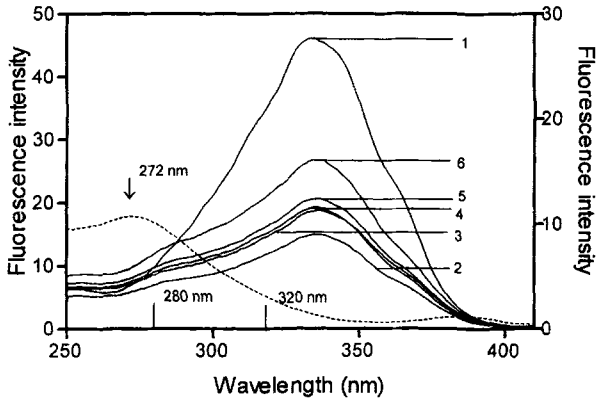

Fig. 11. Fluorescence excitation spectra of RP in binary water/*p*-dioxane solvent registered at different water mole fractions X: curve 1, X = 0.0; curve 2, X = 0.94; curve 3, X = 0.96; curve 4, X = 0.97; curve 5, X = 0.98; curve 6, X = 0.99. Observation at 480 nm, left axis. Dashed line—calculated fluorescence excitation spectrum, right axis. Calculation explained in the text.

absorbed by other centers. That is why the calculated "difference excitation spectrum" does not reflect the real ratios of fluorescence centers existing in the solvent. But it allows the characteristic regions of excitation of these centers to be distinguished. Because of this reason, the excitation spectrum of RP in *p*-dioxane (Fig. 11, curve 1) was normalized to the value of emission intensity (λ_{exc} = 336 nm) of the excitation spectrum of RP in the solvent with $X > 0.9$, and it was subtracted from the excitation spectrum of RP in this solvent. One of the characteristic spectra is shown in Fig. 11 (dashed line). As can be seen, concomitant with "the strong" region of excitation ($\lambda_{exc,1} \approx 320$ nm), a shorter-wavelength band of excitation appears ($\lambda_{exc,3} \approx 280$ nm). A similar region of excitation was observed for RP in the binary solvent water/isopropyl alcohol, $X > 0.9$. This suggests that the RP in binary solvent water/*p*-dioxane ($X > 0.9$) creates CsIII fluorescence centers. The CsIII centers are characterized by an intense region of excitation at $\lambda_{exc} \approx 280$ nm and by the fluorescence spectra in the spectral region of CsI.

*Retinyl Palmitate Self-Organization in Binary Water/Isopropyl Alcohol and Water/*p*-Dioxane Solvents for X > 0.9*

In our previous paper we discussed the mechanism of CsI and CsII creation.[16] Now we focus on the CsIII.

One possible scenario of CsIII creation may be presented in the following way: RP molecules are introduced in a water environment (binary solvent) via nonpolar or low-polarity solvent. In the binary solvent, at $X > 0.9$, RP molecules "have at their disposal" a certain amount of nonpolar molecules. For example, in binary solvent water/*p*-dioxane at X = 0.98 and a RP concentration of $c = 10^{-4}$ M, there are 10^3 dioxane molecules per 1 RP molecule. Hence, RP molecules, as amphiphilic molecules, organize a solvation shell consisting of the required amount of water and *p*-dioxane. The *p*-dioxane molecules are somehow "taken up" from the solvent. However, the water accessibility is rather high. The RP molecules, especially RP molecules in an excited state (RP*), exhibit a high polarizability.[19] This means that, according to the model of the Onsager cavity, the resultant dipole moment of retinol residing in a cavity created in this way may have a much higher value in comparison with the dipole moment of RP in CsI. Such a "polarized" RP* molecule is surrounded by a solvation shell consisting of water molecules and the required number of *p*-dioxane (or isopropyl alcohol) molecules.[20] According to the rule of minimum energy, a specific molecular aggregate is created, e.g., CsIII.

Similarly solvated RP molecules in water/apolar (or water/low-polar) binary solvent with a small water con-

tribution tended to interact between each other and to create rigid structures (CsII). In turbid environments ($0.7 < X < 0.9$) RP molecules tended to create micelles or, eventually, systems with higher degrees of aggregation. This kind of RP interaction in binary solvents with a high water content ($X > 0.9$) is less probable due to the sharp increase in the dielectric constant of the solvent. An increase of the dielectric constant causes, in turn, the force of an electrostatic interaction to decrease (Fig. 1).

The mechanism of CsIII creation presented does not describe the structure of III centers. However, it emphasizes the specific conditions of RP self-organization in binary solvents at $X > 0.9$ and motivates the possibility of a new fluorescence center creation, CsIII centers. Finally, this mechanism explains why RP molecules cause binary solvents of $0.7 < X < 0.9$ to be turbid and binary solvents of $X > 0.9$ to return to transparency.

CONCLUSIONS

The results presented demonstrate that in binary solvents an increase in water content induces a complicated process of RP molecule self-organization: from molecular structures consisting of one or more RP molecules to systems like micelles or microcrystals making the solvent turbid.

The process of RP self-organization was monitored by fluorescence spectroscopy. Hence, the structures appearing were named fluorescence centers. The kind of RP molecular structures depends on the water content of the solvent. CsI was defined as a RP molecule in a solvation shell made of mainly apolar or low-polar solvents. CsI centers exist mostly in *p*-dioxane and isopropyl alcohol. However, they were found in binary solvents of all X values.

An increase in water content induces the selective solvation of RP molecules, leading to dielectric enrichment and to CsII creation. CsII centers are in dynamic equilibrium with CsI. If the water content is not very high, the water dielectric constant does not distinctly influence the resultant dielectric constant of the binary solvent. RP molecules placed in the specific solvation shell may interact so strongly that microcrystals or micelles make the binary solvent turbid ($0.6 < X < 0.9$).

The subsequent increase in water content causes the resultant dielectric constant of the binary solvent to approach the value of the water dielectric constant. As a result, the electrostatic interaction between RP molecules weakens and the appearance of large aggregates becomes less probable. The RP molecules introduced into binary solvents via apolar (or low-polar) solvents "keep" their solvation shells and (if necessary) force the required water molecules to enter these shells. In this way, as a result of self-organization, CsIII centers are created.

This demonstrates that water molecules force RP molecules to build different self-organized structures. Hence, it might be concluded that retinoids may function in water environments as a dispersive phase at concentrations much higher than the value of limited solubility.

The solvents (*p*-dioxane and isopropyl alcohol) were chosen to be different in structure and dielectric properties. Potentially, these solvents themselves may be "organized" and may create different structures with water molecules. That is why different mechanisms of creation RP fluorescence centers were expected. Luckily, the data obtained suggest a similar mechanism for CsI, CsII, and CsIII. This indicates that water molecules play the role of a steering force for different RP self-organized structures. Hence, it is not the solvent used for vitamin A introduction into water that is important, but the vitamin structures induced by water molecules in (always) binary solvents. This finding should be considered by those who investigate retinoid properties and use water as a medium for these studies.

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