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ABSORPTION AND FLUORESCENCE STUDY OF AMYLOSE COMPLEX IN CATIONIC DETERGENT USING BENGAL ROSE AS A SPECTROSCOPIC PROBE

Abstract

In order to estimate the detergent influence on the process of amylose-dye complex formation the absorption and fluorescence studies of Bengal Rose in the amylose – Bengal Rose complex in the presence of cationic detergent, tetradodecyltriethylammonium bromide,TDABr, were carried out. The fluorescence quenching study allowed to calculate binding constant between the dye and amylose. Thermodynamic parameters were calculated from the temperature dependencies of binding constant. Fluorescence lifetime measurements allowed to determine the environment and distribution of the dye in both systems.

An increasing amount of amylose from 0.1% to 2% led to changes in both, absorption and fluorescence spectra. Observed isosbestic point at 623 nm in the absorption spectrum indicated formation of the static complex between amylose and dye. This was confirmed by the fluorescence spectra where a decrease and shift of fluorescence maximum to longer wavelength was observed. When a cationic detergent above its cmc concentration was added to the system with 1.75% amylose and Bengal Rose the fluorescence maximum shifted to 600 nm and its intensity decreased by 5 times as compared with the system without detergent. Calculated enthalpy and entropy had positive values indicating that not only electrostatic processes took place but also hydrophobic forces participated in the complex formation. Calculated stability constant suggested that the detergent facilitated formation of the amylose complex by the factor of 4.

Introduction

An increasing number of ingredients and additives like fooddyes and preservatives [1] as well as environmental impurities modify properties of such significant food component as carbohydrates [2, 3]. The binding complexation of dyes to biopolymers induces changes in the physical properties of dyes. Alterations of the absorption and fluorescence spectra i.e. the fluorescence intensity and emission polarization for many dyes including Bengal Rose were observed [4, 5, 6]. Bengal Rose containing one phenoxide and one carboxylate group, both capable of the reac-

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tion with amylose and/or detergent may readily form complexes serving simultaneously as a probe for the dye environment. Amylose and its derivatives can form complexes with a variety of substrates. It has been found that the major factor in such case is due to the substrate hydrophobicity and solvent polarity [7, 8, 9]. The study indicated that hydrogen bonding between host and guest also influenced the stabilization of the helical form of amylose and determined its reactivity with substrates. The interactions also involved complexed molecule located within or on the helical surface of amylose [10]. In this paper, modification of amylose due to properties complexation by detergent and thermodynamic properties of such complexes were studied using spectroscopic methods.

Materials and methods

The following chemicals were used: Potato amylose from POCH Gliwice (Poland) with DP = 120, as measured by light scattering experiment.

Bengal Rose from SIGMA dissolved in water in the concentration of $1 \cdot 10^{-4}$ M as a stock solution. The dye was recrystallized from methanol before used.

Tetradodecyltriethylammonium bromide TDA-Br detergent from SIGMA. The 28 mM stock solution in water was diluted as requested.

Aqueous samples for absorption and emission studies

Amylose solutions (0.1% to 2%) were prepared by digestion of amylose in water at 80°C for 10 minutes then cooled to room temperature. A required amount of detergent and dye was added to them keeping the total volume of the sample of 1 ml. Prepared samples were measured directly after blending then stored at dark at room temperature for further measurements.

Spectral measurements

The absorption spectra of Bengal Rose were measured using HP4 Photodiode Array Spectrophotometer Hewllet-Packard with the 2 nm resolution.

The fluorescence spectra and fluorescence lifetimes of Bengal Rose and UVCD spectra of amylose were taken using UVCD instrument at National Synchrotron Light Source at Brookhaven National Laboratory, at port U9B [11].

Results

Aqueous absorption and emission spectra of Bengal Rose with amylose

Increasing concentration of amylose, from 0.1% to 1% or, from $5 \cdot 10^{-5}$ to $1 \cdot 10^{-3}$ M, in the presence of $2.5 \cdot 10^{-6}$ M Bengal Rose in aqueous solution led to changes in ab-

sorption and emission spectra. The 549 nm band in the absorption spectrum of Bengal Rose decreased and simultaneously shifted to a longer wavelength. For 1% amylose the peak was located at 554 nm as compared with 548 nm in an aqueous solution. The shoulder located at 514 nm was shifted to 520 nm. The apparent isosbestic point at 623 nm corresponded to the complex formation between amylose and Bengal Rose. The ratio of the absorption intensities at λ_{550} and λ_{514} reached 3, pointing to the monomeric form of the dye in that complex.

The aqueous fluorescence spectra of Bengal Rose with increasing concentration of amylose showed a decrease of fluorescence intensity at maximum and a shift of the maximum to a longer wavelength. For 1% amylose the maximum was at 576 nm compared to 570 nm in water. Decreasing fluorescence intensity reflected a static quenching resulting from the formation of the amylose-Bengal Rose complex, whereas the spectral shift suggested that a part of dye molecules was located in the environment of the dielectric constant lower than for water.

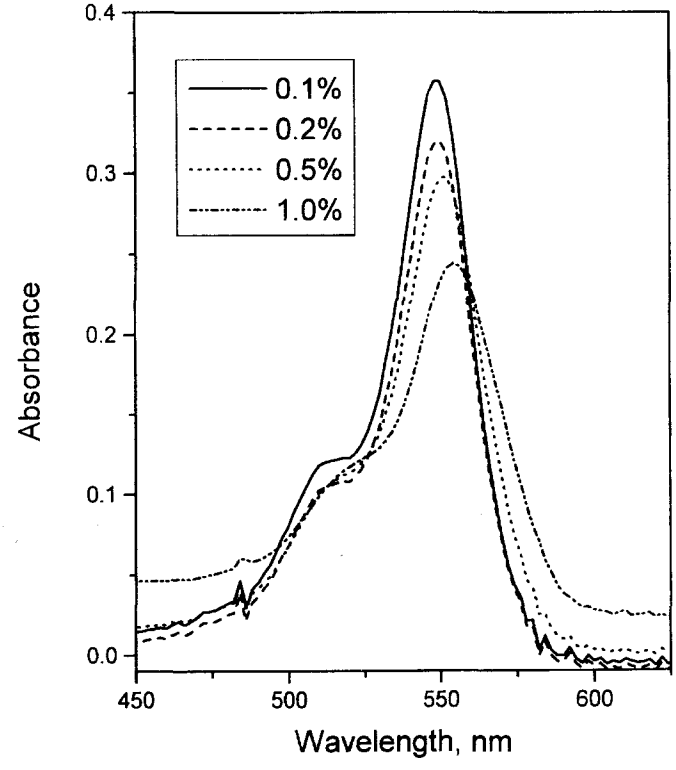

Fig. 1. Absorption spectra of $2.5 \cdot 10^{-6}$ M Bengal Rose in 14 mM detergent, TDABr, versus increasing concentration of amylose. The concentration of amylose is given in the legend.

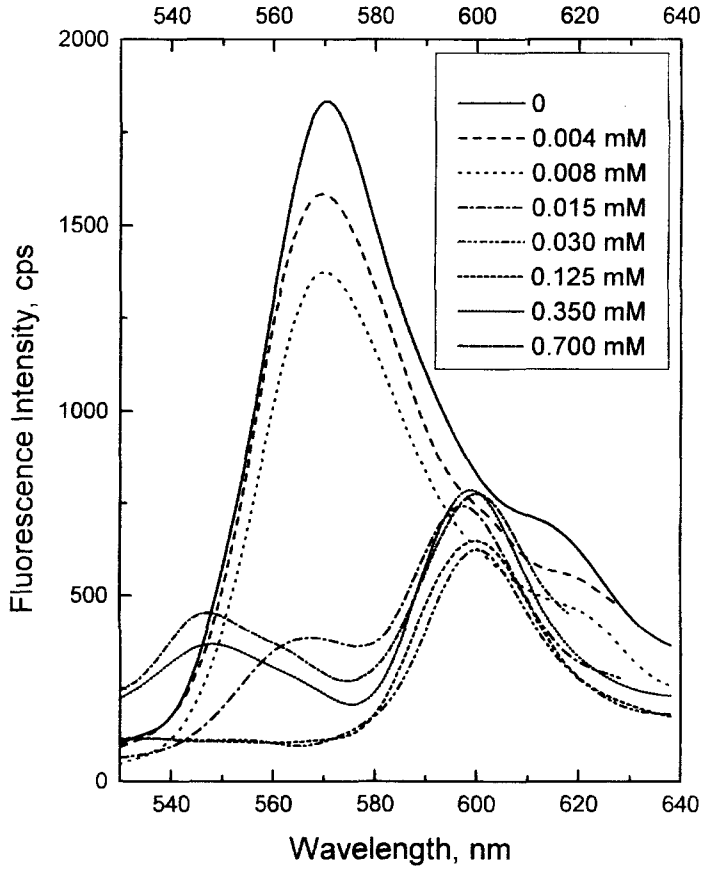

Fig. 2. Fluorescence spectra of $2.5 \cdot 10^{-6}$ M Bengal Rose with 1% amylose at changing detergent, TDABr, concentration, given in the legend, λ_{ex} = 500 nm.

Absorption and fluorescence spectra of Bengal Rose with amylose and TDABr

The absorption and fluorescence spectra of Bengal Rose in the system containing 1.75% amylose, $2 \cdot 10^{-6}$ M Bengal Rose and TDA-Br cationic detergent at the concentration changing from 0.7 μM to 0.7 mM are given in Figures 1 and 2. An increasing amount of detergent moved the absorption peak from 548 nm to a longer wavelength and decreased its intensity. These changes were observed only when the concentration of detergent in the system reached 0.1 mM, i.e. when the detergent concentration reached its critical micelle concentration. The fluorescence spectra of Bengal Rose were also altered. Up to 0.1 mM the increasing amount of TDA-Br quenched the fluorescence at 575 nm. Higher detergent concentration dramatically changed the emission

spectrum of Bengal Rose. The maximum emission was shifted to 600 nm and another peak at 550 nm appeared, the fluorescence intensity decreased by five times as compared with the system without detergent. Color of the solution changed from reddish to light-pink. Such behavior might point to at least two physical processes. The first of them could be the static quenching process of the Bengal Rose emission by amylose and detergent and the second, one could deal with the formation of dimers of Bengal Rose molecules which were incorporated into micelles.

Thermodynamic and kinetic results

Disappearance of the color of the solution as well as observed quenching of the Bengal Rose fluorescence during the complex formation provided spectroscopic determination of the stability constant of such complex k_s. At the ratio of BR/Am higher than 1:20, i.e. with an excess of amylose, we might assume that the host to guest ratio in the complex was 1:1 and for absorbance measurements k_s was given by

$$k_s = [A\text{-}RB]/[A]\cdot[RB] \quad (1)$$

where: $[A]=[A]_o - [A\text{-}RB]$; $[RB] = [RB]_o - [A\text{-}RB]$; [A-RB] – the concentration of the amylose-Bengal Rose complex.

In case of fluorescence measurements we used Stern-Volmer equation to determine that quenching constant, and k_q was given by

$$F_0/F = 1 - k_q\cdot[Q] \quad (2)$$

where: F_0 and F were intensities of the Bengal Rose fluorescence without and with quencher, respectively; [Q] – was the quencher concentration.

Using the fitting program we could obtain the binding constant for both methods. The calculated values were 2460 M^{-1} and 10100 M^{-1} for amylose with Bengal Rose and, for amylose with the Bengal Rose – detergent system, respectively.

In order to calculate thermodynamic parameters of the above systems the fluorescence measurements were carried out at 10°C, 23°C and 40°C. The calculated values for the free energy, ΔG°, enthalpy, ΔH°, and entropy, ΔS°, are presented in Table 1.

The enthalpy of the process was calculated from the slope of the van't Hoff plot. Both enthalpy and entropy had positive values.

Fluorescence lifetime measurements

In order to determine the location and distribution of the dye in that heterogeneous system the fluorescence lifetime measurements were applied. The fluorescence lifetime of Bengal Rose in different systems is given in Table 2. Bengal Rose in water had only one short lifetime at 90 ps. In 1% amylose solution with the ratio of BR/A up

to 1:222 two components were observed. The first, shorter, with 110 ps indicated that the environment of the dye changed to less polar, and the second, the long one, with lifetime of 3.6 ns but contributing only in 10% to the total decay was characteristic for Bengal Rose in the micellar environment. When the concentration of detergent added exceeded 0.1 mM a 3.6 ns component became the major contribution to the observed decay. Third component appearing at 1.7 ns, indicated a lower dielectric constant of the medium and it was observed as a major component when the detergent in that system was below its cmc.

Table 1

Thermodynamic parameters for the complex formation of amylose with detergent and Bengal Rose as calculated from the fluorescence data.

Temp. °C	Binding const $mol^{-1} \cdot 10^{-4}$	ΔG° $kJ \cdot mol^{-1}$	ΔH° $kJ \cdot mol^{-1}$	ΔS° $J \cdot deg^{-1} mol^{-1}$
10	0.34		48.3	92.3
23	1.1	-22		
40	2.8			

Table 2

Fluorescence lifetimes of Bengal Rose in different systems

	lifetime 1		lifetime 2		lifetime 3	
	ps	α	ns	α	ns	α
BR in water	83	1				
BR + amylose[a]	110	0.95			3.6	0.1
BR + am + det[b]	83	0.49			1.7	0.51
BR + am + det[c]	88	0.03	1.7	0.11	3.7	0.88
BR + det[b]	86	0.96			3.7	0.04
BR + det[c]	85	0.81	0.49	0.19		

[a] for amylose concentration from 0.1% to 2%,
[b] bellow cmc of detergent,
[c] above cmc of detergent.

Detergent concentration study

As indicated by the data in Table 2 it was obvious that the presence of amylose changed the micellization process of the dye. In order to confirm that amylose influ-

enced the detergent cmc the experiments with and without amylose in the system were carried out. The results are presented in Fig. 3. It is clear from this Figure that the presence of amylose in the detergent solution decreased its cmc by more than one order of magnitude from original 1.4 mM to **8** mM. In that case we might suggest that amylose served as a condensation center for micelization what pointed to more dye molecules located in the micellar environment.

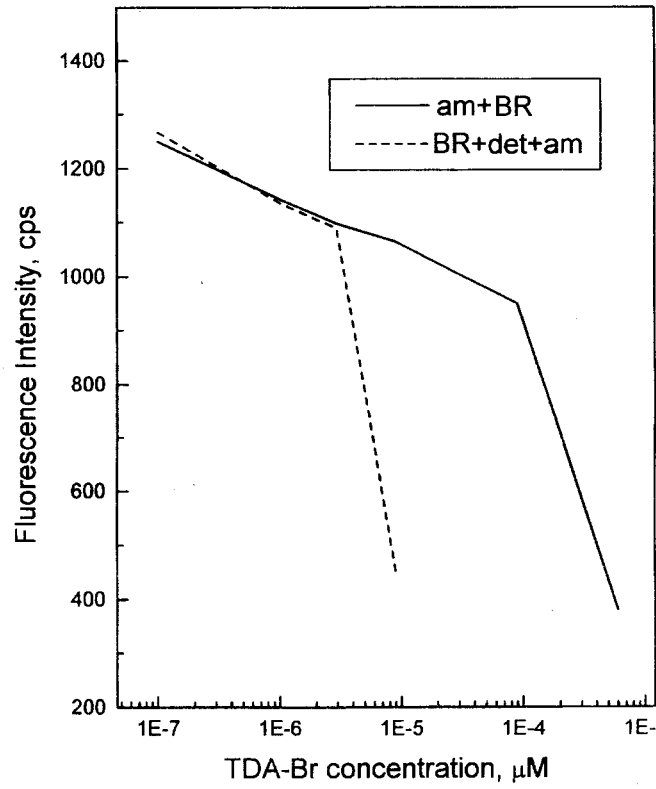

Fig. 3. Fluorescence intensity of $2 \cdot 10^{-6}$ M Bengal Rose without and with 1% amylose versus detergent concentration

Discussion

In the present study the influence of the cationic detergent on the complexation process between amylose and dye was studied. Our previous papers [**8**, 9, 12] showed that Bengal Rose and amylose chain either formed an inclusion complex or adsorbed on the amylose surface. When cationic detergent was added further changes in the system were observed. Absorbance and fluorescence study showed generally decreasing trend with increasing concentration of amylose and detergent. Additionally, a red

shift relative to aqueous system occured. All those changes delivered a strong evidence for the complex formation. The stability constant for the amylose-Bengal Rose complex formation, k_q, was 2460 M^{-1}. In the presence of detergent the stability constant was 10100 m^{-1}. It means, that detergent facilitated the complex formation by the factor of 4. The influence of the detergents on the amylose behavior followed a general mechanism where the formation of inclusion complexes involved hydrocarbons [13]. This fact might have serious implications if we assumed that any other detergent-like hydrocarbons which interacted with carbohydrates might increase the ligand uptake like heavy-ion metals or other pollutants.

Whereas a liner Stern-Volmer plot generally indicated an equivalent accessibility of all species of fluorophores present in particular system to the quencher, the exact mode of quenching it is not always clear. In that case contributions from both static and dynamic quenching should be considered. Temperature study was performed to distinguish between two possible quenching. The temperature increase in the range from 10 to 40°C in both cases was paralleled by the S-V quenching constant increase. It suggested a dynamic quenching resulting from increased collisional deactivation. Fluorescence lifetime measurements appeared to be a sensitive tool in resolving the exact contribution of dynamic component. The plot of preexponential factors or the lifetime ratio versus the amylose concentration should give a slope equal to kq, kd and ks quenching constants. Calculated dynamic part was about 20% in both cases and the static part contributed largely to the overall quenching process. Results of this study confirmed the contribution from the static quenching.

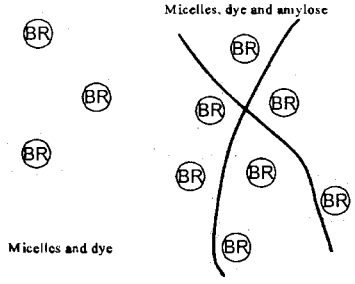

Fig. 4. Schematic representation of the micellization process under absence and presence of amylose.

Detergent concentration study showed that the cationic detergent facilitated formation of the amylose-Bengal Rose complex. Additionally, we might rationalize this process in terms of the detergent induced additional micelisation of the system, i.e. the amylose helix served as a chain around of which the miceles were formed. Such bind-

ing in solution might be considered as a consequence of the rigidity loss of the amylose molecules caused by the detergent (see Fig. 4).

These considerations are also supported by thermodynamic parameters given in Table 2. Positive enthalpy value indicated that the hydrogen bonding appeared to be unfavorable and the positive entropy effect indicated the formation of a hydrophobic bond in the system. This might be attributed to the release of the hydrated water molecules from amylose upon binding. Thermodynamic data indicated that the driving force for the dye complexation to amylose in aqueous and micelar solution were electrostatic as well as hydrophobic.

Acknowledgment

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REFERENCES

[1] Heijnen M.L., van Amelsvoort J.M., Weststrate J.A.: Eur. J. Clin. Nutr., **49**, 1995, 446-57.
[2] Wulff G., Kubik S.: Carbohydr. Res., **237**, 1992, 1-10.
[3] Tezuka Y.: Biopolymers, **34**, 1994, 1477-81.
[4] Neckers D.C.: J. Photochem. Photobiol., Ser. A, **47**, 1989, 1-29.
[5] Rodgers M.A.: J. Chem. Phys. Letters, **76**, 1981, 509-514.
[6] Rodgers M.A.: J. Phys. Chem., **85**, 1981, 3372-3374.
[7] Hui Y., Gai Y.: Makromol. Chem., **189**, 1988, 1281-1294.
[8] Polewski K., Maciejewska W.: Carbohyd. Res., **226**, 1991, 179-183.
[9] Maciejewska W., Polewski K., Wyspiańska-Grunwald M.: Carbohyd. Res., **246**, 1993, 243-251.
[10] Jiang X.K., Li X.Y., Huang B.Z.: Proc. Indian Acad. Sci. (Chem. Sci.), **98**, 1987, 423-429.
[11] Polewski K., Kramer S.L., Kolber Z.S., Trunk J.G., Monteleone D.C., Sutherland J.C.: Rev. Sci. Instrum., **65**, 1994, 2562-2567.
[12] Polewski K., Maciejewska W.: Carbohyd. Res., **246**, 1993, 253-265.
[13] Bulpin P.V., Cutler N.A., Lips A.: Macromolecules, **20**, 1987, 44-49.

POMIARY ABSORPCJI I FLUORESCENCJI KOMPLEKSÓW AMYLOZY W DETERGENCIE KATIONOWYM UŻYWAJĄC RÓŻU BENGALSKIEGO JAKO SONDY FLUORESCENCYJNEJ

Streszczenie

W celu określenia wpływu detergentów na proces powstawania kompleksu amyloza-barwnik przeprowadzono pomiary absorpcji oraz fluorescencji różu bengalskiego w obecności kationowego detergenta bromku tetradodecyltriethyloammoniowego, TDABr. Pomiary wygaszania fluorescencji różu bengalskiego pozwoliły wyznaczyć stałą tworzenia kompleksu między barwnikiem a amylozą. Parametry termodynamiczne obliczono z temperaturowych zależności stałych tworzenia kompleksu. Pomiary czasów życia fluorescencji pozwoliły na określenie środowiska oraz rozkładu barwnika w badanym układzie.

Ilość amylozy wzrastająca od 0.1% do 2% prowadzi do zmian w widmach absorpcji i fluorescencji barwnika. Pojawienie się przy 623 nm punktu izosbestycznego wskauje na powstanie kompleksu pomiędzy amylozą a barwnikiem. Ten proces zostaje potwierdzony w pomiarach widm fluorescencji, w których obserwuje się zmniejszenie natężenia oraz przesunięcie maksimum w stronę fal dłuższych. Dodanie kationowego detergenta do układu 1.75% amylozy z barwnikiem powoduje, że maksimum fluorescencji przesuwa się do 600 nm, a natężenie emisji zmniejsza się pięciokrotnie w porównaniu z układem bez detergenta. Obliczone wartości entalpii i entropii mają dodatnie wartości co stanowi wskazówkę, że oprócz procesów elektrostatycznych w tworzeniu kompleksu uczestniczą także oddziaływania hydrofobowe. Obliczone stałe wskazują, że obecność detergentu czterokrotnie ułatwia tworzenie kompleksu z amylozą.