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FLUORESCENCE AND ORD STUDY OF AMYLOSE-DYE COMPLEX

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Fluorescence polarization and optical rotatory dispersion (ORD) techniques were used to investigate the formation of amylose-rose bengal complexes. It was found that the dye may intercalate amylose helixes and adsorb on the amylose surface. An ORD measurements also provided information that addition of a rose bengal to the amylose aqueous solution was responsible for the formation of a lefthanded helixes in amylose chain.

INTRODUCTION

Amylose can form helical inclusion complexes with a variety of substrates. The study of this complexes has received interesting attention [1-6]. A full understanding of the aqueous complexes has been elusive mainly because in this case the complexes may exist in various stages of aggregation and various conformations of amylose chain [5, 7, 8]. Recently it has been reported that amylose in diluted aqueous solutions, in the absence of complexing agents, may take one of three kinds of form: a random coil, a so-called interrupted helix and a deformed helix [9]. It is also noteworthy to point out that local conformation of amylose chain is very important for the formation of helical inclusion complexes [6].

The aim of this work is to study the formation of amylose-rose bengal complexes. The conditions of formation the complexes were analyzed by the fluorescence polarization method (FPM). The content of the helix form in complexed amylose chain was determined from optical rotatory dispersion (ORD) measurements.

MATERIALS AND METHODS

Potato amylose obtained from Polish Chemical Reagents was used. Amylose powder was digested for 30 minutes in boiling doubly destilled water. Next the solution was cooled down to 25°C. Amylose from stock solution was mixed with

solution of rose bengal in desired ratio. In the case of fluorescence measurements the concentration of rose bengal, X, was constant and equal to 2×10^{-5}M, whereas the amylose concentration, C_a, was varied from 0.1% to 6% (m/V) (the corresponding molar fractions of amylose were from 0.12×10^{-4} to 5.20×10^{-4}). In the case of ORD measurements the amylose concentration was constant, equal to 0.9% and rose bengal concentration was varied from 2×10^{-5} to 5.2×10^{-4}M. ORD of amylose-rose bengal complex was measured at five wavelengths, from 366 nm to 578 nm, with POLMAT A polarimeter, using 2 dcm cell. The fluorescence polarization spectra were measured with a typical righ angle set up. A light from Xe 150 W xenon lamp passes through interference filter IF 525 nm and polarizer and hits a sample. Emission light through emission monochromator analyzer falls on the PMT FEU-79, S-20 type photocathode.

RESULTS AND DISCUSSION

FLUORESCENCE POLARIZATION STUDY (FPS)
FLUORESCENCE STUDY RESULTS ARE SUMMARIZED IN TABLE 1.

The first band at 560 nm describes a behaviour of that part of dye molecules which intercalate or genarally saying those ones which happen to occur in hydrophobic environment like e.g. the interior of the amylose chain. The other band at 585 nm is quenched as the concentration of amylose increses that clearly indicates the formation of nonfluorescent amylose-rose bengal complex. FPS is based on the fact that fluorescence of the dye which is adsorbed on the macromolecule partially polarized, while a fluorescene of free dye is usually unpolarized. Therefore in any mixture of a fluorescent dye with a macromolecule where part of dye is bound to the macromolecule, the observed polarization P will be less than that for completely bound dye, P_B. Thus the value $p = P/P_B$ may serve as measure of fraction of a dye bound to a macromolecule [10].

Table 1. The fluorescence emission intensities of rose bengal at different amylose concentration, rose bengal 2×10^{-5}M

amylose	I_{560}	I_{585}
0.1%	425	345
0.5%	580	260
1.0%	960	115
3.0%	2100	5
5.0%	5000	n.m.
8.0%	6800	n.m.

n.m. — not measurable

Table 2. Fraction of dye molecules, z, bounded within cavity of amylose helixes

$C_a \times 10^{-4}$ [M]	0.12	0.20	0.70	1.40	1.50
z	0.20	0.27	0.30	0.38	0.55

We have found that the rose bengal molecules can bind to amylose in two forms. In the first form — the rose bengal molecules are adsorbed on amylose surface that leads to the formation of nonfluorescent complex. In the second — the dye molecules can form intercalative binding resulting in formation of the fluorescent helical inclusion complex.

To calculate the number of adsorbed dye molecules per amylose molecule segment, n, fraction of bounded dye molecules, z, and equilibrium constant, K, we used the modified Klotz equation [10], namely:

$$K = nC_{\alpha}(1-z)z^{-1} \tag{1}$$

where C_{α} is amylose concentration.
Fraction of a dye bounded within amylose helixes is determined as:

$$z = pR-(R+1) \tag{2}$$

where R is fluorescence intensity ratio of dye-amylose complex to the free dye emission.

We have found that the values of K and n are insensitive to the amylose concentration and they are equal to: $K = 2.3 \times 10^{-4}$ M and $n = 1/3$. The obtained values of z, as a function of amylose concentration are given in Table 2.

The obtained changes of the fraction of a bounded dye molecule with simultaneously constant number of dye molecules adsorbed per amylose segment implie on the increase of helical forms in amylose chain. In order to confirm this suggestion we used on ORD spectroscopy, because the value of molecular rotation depends on the fraction of the helix form in the polymer chain.

OPTICAL ROTATORY DISPERSION STUDY

An ORD method was applied to investigate the conformational transitions induced in amylose by a complexing rose bengal molecules. Our studies of the optical rotatory dispersion behavior of amylose solutions in the presence of rose bengal have shown that a plateau region of molecular rotation ($[m]_{\lambda}$), as a function of a rose bengal concentration, exists at high concentrations, (Fig. 1). It enables us to calculate the approximate linear measure of the fraction of the helix forms in amylose chain, f_H, according to the formula proposed in [11]:

$$f_H = ([m_w]_{\lambda} - [m]_{\lambda})([m_w]_{\lambda} - [m_p]_{\lambda})^{-1}, \tag{3}$$

where $[m_w]_{\lambda}$ is the molecular rotation of amylose in pure aqueous solution and $[m_p]_{\lambda}$ is the plateau value at high rose bengal concentration. The obtained of f_H are presented in Fig. 2. The shape of the curve indicates that the increase of the concentration of rose bengal in the studies amylose solutions yields the increase

of the content of the helix forms in amylose chain. But when the concentration of dye is higher than 1.2×10^{-5} M the plateau region has been observed.

Moreover, the shape of the function $[m]_\lambda$ (Fig. 1) gives the details on the chirality of the helixes. Namely, when the formed helixes are lefthanded it corresponds to the increase in the molecular rotation whereas the formation of righthanded helixes corresponds to the decrease of the molecular rotation. In our case $[m]_\lambda$ increases with increase of rose bengal concentration what leads to the conclusion that lefthanded helixes are preferred in amylose-rose bengal complexes.

CONCLUSIONS

The analysis of fluorescence results leads to the conclusion that rose bengal molecules can be both adsorbed on amylose surface and bounded within cavity

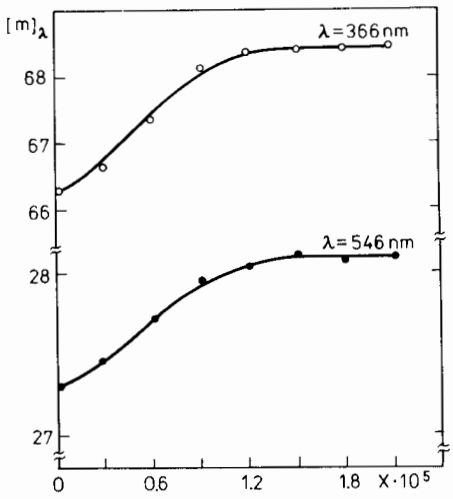

Fig. 1. Dependence of molecular rotation, $[m]_\lambda$, (in deg cm^{2}mol^{-1}) of amylose at 0.9% (w/V) on rose bengal concentration, X, (in M)

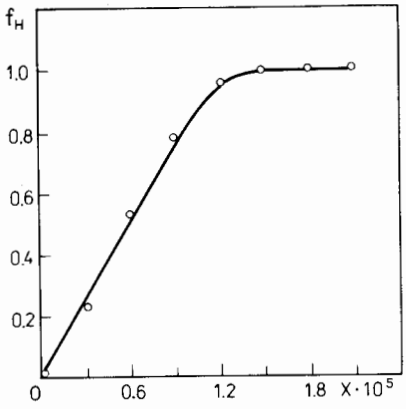

Fig. 2. Content of the helix form, f_H, in amylose-rose bengal complexes *vs* the rose bengal concentration

of amylose helixes. The number of adsorbed dye molecules per amylose molecule segment is insensitive to the amylose concentration and a fraction of dye bounded to amylose helixes varies with concentration from 0.20 to 0.55 in the studied region.

The application of ORD spectroscopy gives the possibility of finding the content and chirality of helix forms in amylose-rose bengal complexes. We have found that the addition of dye molecules to amylose solutions in responsible for the formation of lefthanded helixes in amylose chain.

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STUDIA NAD KOMPLEKSEM AMYLOZA-BARWNIK METODAMI POLARYZACJI FLUORESCENCYJNEJ I DYSPERSJI SKRĘCALNOŚCI OPTYCZNEJ (ORD)

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Streszczenie

Metodami spektroskopii fluorescencyjno-polaryzacyjnej (FPM) i dyspersji skręcalności optycznej badano tworzenie się kompleksu amylozy i różu bengalskiego. Stwierdzono, że cząsteczki różu mogą przyłączać się do amylozy w dwojaki sposób: mogą one być adsorbowane na powierzchni amylozy lub tworzyć wiązanie na zasadzie wtrącenia (interkolacyjne) podczas tworzenia inkluzyjnego kompleksu helisy. Znaleziono, że wartość stałej równowagi „K" i liczba „n" zaadsorbowanych

cząsteczek barwnika na 1 segment amylozy są niezależne od stężenia amylozy w badanym obszarze. Otrzymane zmiany ułamka związanego barwnika z jednoczesną stałą wartością „n" wskazują na wzrost formy helikalnej w łańcuchu amylozowym. Badania ORD dostarczyły informacji o chiralności i frakcji helisy w łańcuchu amylozy. Obliczenia parametrów Moffita wskazują, że dodawanie różu bengalskiego do wodnego roztworu amylozy powoduje tworzenie formy lewoskrętnej helisy amylozy.