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FT-IR and Raman spectroscopy study of the amylose–Rose Bengal complex *

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ABSTRACT

FT-IR and Raman spectra of amylose–Rose Bengal in solution and in lyophilised samples were investigated. In the Rose Bengal molecule, the number and positions of active atoms, i.e., those that interact with amylose, were determined. The interaction with amylose was found not to affect the Rose Bengal quinonoidal structure. Results for the amylose complex show considerable hindrance of the rotational vibrations of amylose functional groups. Moreover, the influence of Rose Bengal on the infrared signals in the structure-sensitive region (950–1200 cm^{-1}) was studied. The FT-IR results indicated the formation of a stiff amylose network induced by Rose Bengal molecules.

INTRODUCTION

Amylose can form helical inclusion complexes with a variety of molecules, provided that they have a suitable diameter to fit within the helix[1–8]. A full understanding of the dissolved complexes has been elusive, mainly because of the large number of conformational states of the amylose chain and because the complexes may exist in various states of aggregation[2,9–12]. Furthermore, the local conformation of the amylose chain (random coil, deformed helix, or interrupted helix) is also a matter of dispute[13–18]. Apart from helical inclusion complexes, another mechanism of complex formation, as in the case of the carbohydrate–protein interaction, can be observed[19]. The effects of interaction between carbohydrate and protein chains lead to the formation of a liquid- or gel-like phase via a process known as complex coacervation.

From the technological point of view, amylose seems to be an appropriate polymer for dye complexing[20]. However, until now, only a few papers on amylose–

* Amylose–Rose Bengal Complexes, Part II. For Part I, see ref 28.
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dye complexes have been published and they have concerned only the helical inclusion complexes[8].

In our previous papers[21,28], we presented a spectroscopic study of the amylose–Rose Bengal complex and proposed two possible mechanisms for complex formation. Rose Bengal has been chosen as a model dye because of its characteristic properties[22]; its functional groups could be appropriate for interaction with amylose (Fig. 1). Furthermore, Rose Bengal has strong spectroscopic lines, convenient for interpretation[23]. We suggested[21] that Rose Bengal molecules may bind to amylose through the C=O bond in the C-3 position.

It is known that the formation of complexes between amylose and other molecules leads to changes in the amylose chain conformation and in the structure of the whole amylose sample[2,7,19]. In the previous paper[21], we reported an increase in the helix form with an increasing amount of Rose Bengal in the solution. Simultaneously, we observed, in the structure-sensitive region 950–1200 cm^{-1} of the IR spectrum, a new line at 1124 cm^{-1}, which may be assigned to the related modes of C–H, C–OH deformation, and C–O–C antisymmetric stretching vibrations.

In Part I[28], we suggested two possible mechanisms of amylose and Rose Bengal complex formation: by helical inclusion and adsorption on to an amylose chain. However, on the basis of the data available, we could not decide which one of them dominates. The aim of this work was to determine precisely the path of complex formation between amylose and Rose Bengal as well as to define the atoms and atomic groups in the molecules of these compounds involved in the process of complex formation.

EXPERIMENTAL

Preparation of amylose–Rose Bengal solutions.—Rose Bengal (sodium salt, Fig. 1) was purchased from Sigma, dissolved in doubly distilled, deionised water to an appropriate concentration (varying from 2×10^{-1} to 2×10^{-6} M). Potato amylose (dp 120, as indicated by light scattering) was obtained from Poland Chemical Reagents. Solutions prepared by digesting amylose powder for 20 min in boiling water were cooled to 25°C at 12°C/min and mixed with a solution of Rose Bengal

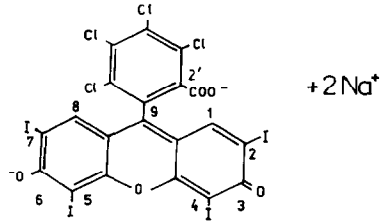

Fig. 1. Molecular structure of the dianionic form of Rose Bengal in water solution.

to obtain the desired concentration. In order to obtain lyophilised samples, the mixtures were frozen and then lyophilised for 30 h. The Raman measurements were made for an amylose–Rose Bengal solution of 1×10^{-3} M Rose Bengal (C_{RB}) and 5% (w/v) amylose (C_a) concentrations. For the FT-IR measurements in the solutions, C_{RB} was varied in the 4×10^{-2}–4×10^{-6} M range and the amylose concentration C_a was 2%. For FT-IR measurements on the lyophilised samples, C_{RB} was 4×10^{-2} M and C_a was varied in the 0.5–20% range. Thus, for the lyophilised samples, the number of Rose Bengal molecules per amylose molecule (X) was varied from 4 to 160.

Spectroscopy.—The FT-IR spectra for the solutions (950–1500 cm^{-1}) were recorded with a Bruker IFS 113v spectrometer [2 cm^{-1} resolution and KRS5 (6 μm) cell]. FT-IR spectra for lyophilised amylose were recorded for KBr pellets.

The Raman spectra were recorded with a Spex Model 1877 Triplemate double monochromator equipped with 1200 groove/mm holographic gratings. The samples were excited with the 634-nm line of a Spectra Physics Model 2020 krypton ion laser; the power was 250 mW at the sample. The spectra were taken at room temperature and samples were stirred prior to measurements. The detection system was a Model 1421 intensified diode array (EG&G Princeton Applied Research Corp.) used with a Model 1461 detector interface.

RESULTS AND DISCUSSION

The FT-IR results for Rose Bengal complexes with amylose (Fig. 2) indicate changes in the integral intensities of the bands at 1341 (C-6–O and CO_2^- symmetric stretching) and 1447 cm^{-1} (the aromatic ring, in-plane bending, β $C_{AR}C_{AR}$ vibrations). The integral intensity of the 1341 cm^{-1} band (with respect to the 1447 cm^{-1} band) changed from 0.76 in pure water to 1.08 in 5% amylose (Fig.

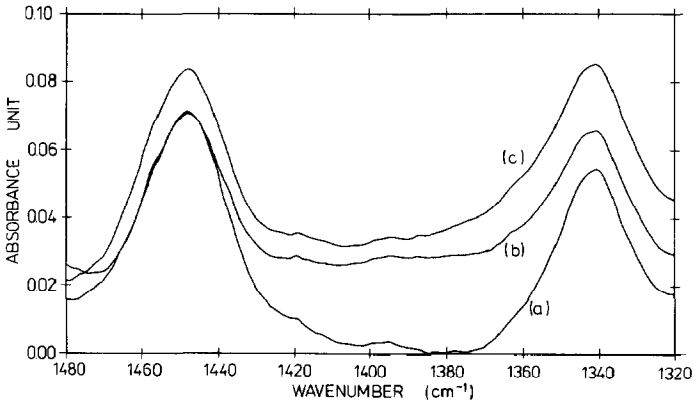

Fig. 2. FT-IR spectra of 4×10^{-2} M Rose Bengal in (a) water solution, (b) aq 1% amylose, and (c) aq 5% amylose.

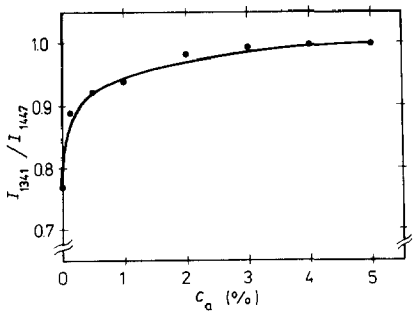

Fig. 3. The integral intensity of the 1341 cm^{-1} IR line (with respect to the 1447 cm^{-1} line) for 5×10^{-2} M Rose Bengal in amylose solution; the amylose concentration (C_a) was varied from 0.1 to 5%.

3). The increase of the 1341 cm^{-1} band intensity may be due to the appearance of new β OH (OH in-plane deformation) and ν (C–OH stretching) vibrations. In general, bands appearing in the 1250–1410 cm^{-1} region have both β OH and ν C–OH vibration character, but β OH vibrations predominate[24a]. The changes observed in the 1341 and 1447 cm^{-1} band integral intensities enable us to conclude that Rose Bengal molecules bind amylose through oxygen atoms at positions 3 and 6, and the carboxyl group at C-2′ (see Fig. 1).

It is possible that Rose Bengal interacts with amylose through its chlorine and iodine atoms. Unfortunately, the carbon–chlorine and carbon–iodine stretching vibrations (800–550 and 600–500 cm^{-1} regions) overlap with strong water absorption bands. Therefore, in order to determine the participation of chlorine and iodine atoms in complex formation, we applied the Raman spectroscopy method. The frequencies and intensities of the Raman spectrum bands of Rose Bengal in water and in aqueous 5% amylose solution are presented in Table I. There are some differences between the spectrum of Rose Bengal alone and that with amylose. We found new bands at 1421, 1198, and 680 cm^{-1} (see Table I). According to the data reported by Colthup et al.[25], these bands may be assigned as follows: 1421, CH_2OH deformation; 1198, C–C–O asymmetric stretching; and 680 cm^{-1}, C–OH bending vibrations. Other differences are observed in the 650–850 and 350–400 cm^{-1} spectral regions, where the C–Cl–O, C–Cl, and H–Cl stretching vibration bands occur (Fig. 4). The band at 730 cm^{-1} (peak 24) may be assigned to the antisymmetric C–Cl–O stretching vibration. The band observed at 756 cm^{-1} (peak 23) may be attributed to the C–Cl stretching vibration when the Cl atom is attached to an α-D-glucopyranosyl residue of amylose at the axial position, whereas the band at 795 cm^{-1} (peak 22) may be attributed to the case when this Cl atom is attached at the equatorial position[24b]. For Rose Bengal in 5% amylose solution, we observed a real increase of the 795 cm^{-1} band intensity and a decrease of the 756 cm^{-1} band intensity relative to the intensity of this band in water. Moreover, we found an increase of intensities of the 830 and 730 cm^{-1} bands. The changes occurring in the 650–850 cm^{-1} region correspond to that in

TABLE I

Positions and intensities in the Raman spectra of Rose Bengal in solution

Peak	Wavenumber (cm^{-1})	Intensity
1	1612	v. strong
2	1546	strong
3	1524	weak
4	1487	v. strong
5	1458	weak
6	1421 with amylose only	v. weak
7	1340	medium
8	1292	strong
9	1267	strong
10	1237	v. weak
11	1198 with amylose only	weak
12	1160	weak
13	1136	weak
14	1044	weak
15	1010	strong
16	979	weak
17	954	weak
18	893	weak
19	866	v. weak
20	862	v. weak
21	830	weak
22	795	weak
23	756	medium
24	730	weak
25	710	medium
26	692	strong
27	680 with amylose only	weak
28	670	v. weak
29	629	shoulder
30	612	v. strong
31	569	strong
32	530	weak
33	518	weak
34	490	weak
35	452	weak
36	426	weak
37	390	medium
38	371	medium
39	344	strong
40	330	strong
41	310	strong
42	252	strong

the 340–400 cm^{-1} region (H–Cl stretching), i.e., the introduction of amylose molecules into the Rose Bengal solution induced a decrease of the 344 (peak 39) and 390 cm^{-1} (peak 37) band intensities (Fig. 4). The changes in the Raman spectrum of a Rose Bengal solution induced by amylose molecules lead to the conclusion that the Rose Bengal chlorine atoms are combined with amylose. On

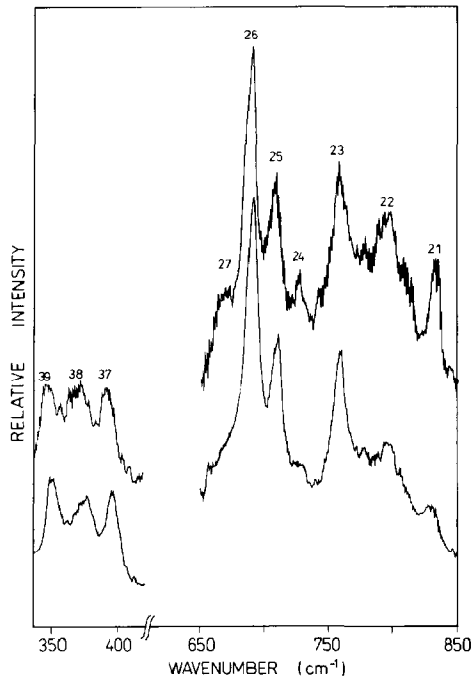

Fig. 4. Raman spectra (in the 650–850 and 350–400 cm^{-1} regions) of 1×10^{-3} M Rose Bengal in (upper trace) water solution and (lower trace) aq 5% amylose.

the other hand, the changes in the 500–600 cm^{-1} region, where the C–I vibrations (526 and 571 cm^{-1} bands) occur, are not observed, which may indicate that iodine atoms do not participate in the complex formation.

To pinpoint the influence of Rose Bengal on the FT-IR spectra of amylose in solution, we selected for consideration two spectral parameters in the structure-sensitive 950–1200 cm^{-1} region of the spectrum. These are the half band-widths and the integral intensities of the 1026, 1082, and 1154 cm^{-1} bands. For amylose in a Rose Bengal solution, a small increase of the 1026 and 1154 cm^{-1} half band-widths with increasing concentration of Rose Bengal is observed, whereas the 1082 cm^{-1} half band-width remains constant. Therefore, we compared the changes of the 1026 and 1154 cm^{-1} band integral intensities relative to the intensity of the 1082 cm^{-1} band (Fig. 5). Comparing the shapes of the curves in Fig. 5, we found that the 1026 cm^{-1} band is more sensitive to the presence of Rose Bengal molecules in the solution than the 1154 cm^{-1} band. Taking into account the vibrational assignments for these bands, 1026 (C–OH bending) and 1154 cm^{-1} (C–O, C–C stretching, and C–H deformation), we can say that the C–OH bending vibrations are very sensitive to interaction with Rose Bengal molecules. Thus, it seems that even a very small amount of Rose Bengal in amylose solution changes the vibrations of C–OH because of its connection with the *gauche* and/or *trans* orientation of a long CH_2OH functional group[7,26].

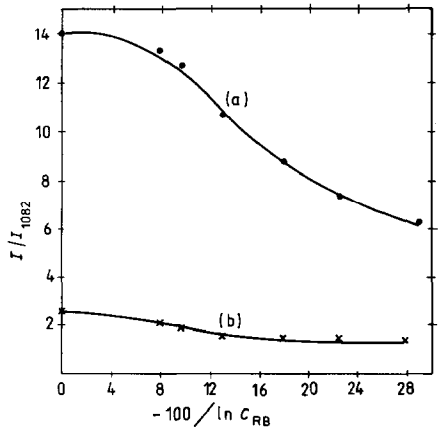

Fig. 5. The integral intensity of the 1026 (a) and 1154 cm^{-1} (b) IR lines (with respect to the 1082 cm^{-1} line) for 2% of amylose in the solution vs. concentration of Rose Bengal (C_{RB}).

In order to obtain a better insight into the mechanism of amylose–Rose Bengal complex formation, we investigated the FT-IR spectra in the 400–4000 cm^{-1} region, for amylose lyophilised from mixtures of various amylose and Rose Bengal concentrations. The application of lyophilised samples made it possible to extend considerably the concentration ranges of the amylose and Rose Bengal. The amylose and Rose Bengal concentrations in the lyophilised mixtures varied from 0.5 to 20% (e.g., from 2.5×10^{-4} to 1×10^{-2} M) and from 2×10^{-5} to 2×10^{-1} M, respectively. The FT-IR spectra of lyophilised Rose Bengal, alone and with amylose, show a strong band at 1608 cm^{-1}, indicating that the Rose Bengal molecule has a quinonoidal structure in both states[27].

The FT-IR spectra of lyophilised amylose are presented separately for the 2600–3800, 950–1200, and 800–950 cm^{-1} regions (Figs. 6–10). Analysis of the OH stretching vibrations (3000–3600 cm^{-1}) is very difficult because of the occurrence of different OH groups in the sample, both intra- and inter-molecularly bonded. Absorption by crystal and structural water is another complicating factor in this analysis.

In the 2800–3000 cm^{-1} region, three bands [2942 (CH groups), 2874 (CH_2 groups), and 2830 (sh) cm^{-1} (CH in CH_2OH groups)] have been observed for pure amylose (Fig. 6). The presence of Rose Bengal molecules gives rise to the disappearance of the 2830 and 2874 cm^{-1} bands and to a slight decrease of the 2942 cm^{-1} band intensity. In polysaccharides, the spectrum in the 2800–3600 cm^{-1} region (OH and CH stretching) could be compared with that in the 1500–1250 and 950–800 cm^{-1} (OH and CH deformation) regions. Unfortunately, in our case, in the 1500–1250 cm^{-1} region, there are strong lines due to Rose Bengal itself[23], and it is difficult to observe the important CH_2OH group. Because of this, we can analyse only two band assignments, OH and CH vibrations 930 (OH bending) and 856 cm^{-1} (C-1–H bending in the α configuration) (Fig. 7). For the amylose

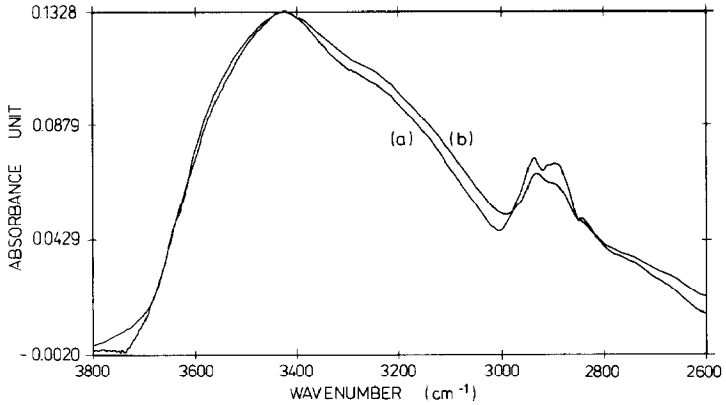

Fig. 6. FT-IR spectra in the 2600–3800 cm^{-1} region of lyophilised samples for (a) pure 5% amylose and (b) 5% amylose in 4×10^{-2} M Rose Bengal ($X = 16$ Rose Bengal molecules per amylose molecule).

complex with Rose Bengal, the 930 and 856 cm^{-1} band intensitics decrease with increasing concentration of Rose Bengal; for the composition equal to 80 Rose Bengal molecules per amylose molecule, these bands disappear. A decrease of the 930 and 856 cm^{-1} band intensities may indicate a considerable hindrance to the OH and CH rotational vibrations; when all amylose OH and CH groups are joined with appropriate Rose Bengal atoms, the 930 and 856 cm^{-1} bands are not observed. Simultaneously, the position of the 930 cm^{-1} band (for pure amylose) shifts to 911 cm^{-1} for the composition of 80 Rose Bengal molecules per amylose molecule. The possible explanation of the band shift is that the formation of a bond to Rose Bengal through the hydrogen atom decreases the bond density in the O–H bond, therefore decreasing its rotational frequency.

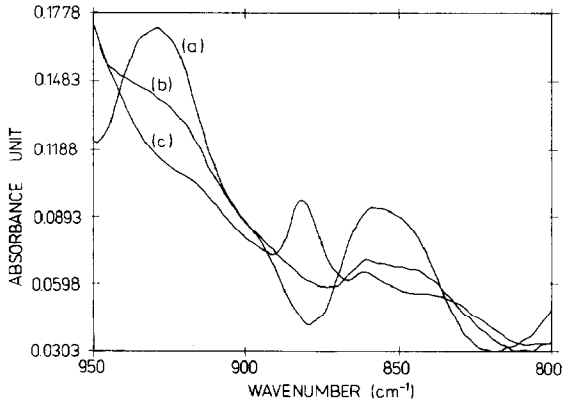

Fig. 7. FT-IR spectra in the 800–950 cm^{-1} region of lyophilised samples for (a) pure 10% amylose, (b) 10% amylose in 4×10^{-3} M Rose Bengal ($X = 8$ Rose Bengal molecules per amylose molecule), and (c) 10% amylose in 4×10^{-1} M Rose Bengal ($X = 80$ Rose Bengal molecules per amylose molecule).

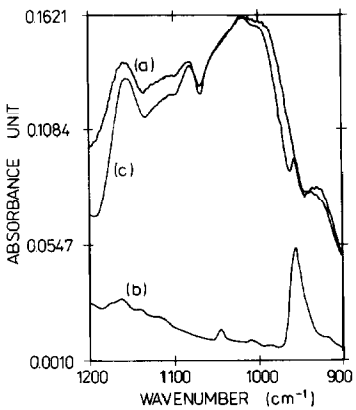

Fig. 8. FT-IR spectra in the 950–1200 cm^{-1} region of lyophilised samples for (a) pure 20% amylose, (b) pure 4×10^{-2} M Rose Bengal, and (c) 20% amylose in 4×10^{-2} M Rose Bengal ($X = 4$ Rose Bengal molecules per amylose molecule).

Infrared spectra in the 950–1200 cm^{-1} region can be used effectively for monitoring structural changes in amylose samples induced by Rose Bengal molecules. For pure amylose, the three bands 1024 (C–H deformation), 1080 (C–H, C–OH deformation, and C–O–C asymmetric stretching), and 1154 cm^{-1} (C–O, C–C stretching, and C–H deformation) appear in this region. We observed some differences in the shape of the spectra obtained for the samples of different amylose concentration in the lyophilised mixtures. For this reason, the spectra of complexes of the three selected amylose concentrations (0.5, 2, and 20%) and a constant Rose Bengal concentration (equal to 4×10^{-2} M) have been analysed (Figs. 8–10). During analysis of the changes in amylose spectra induced by Rose

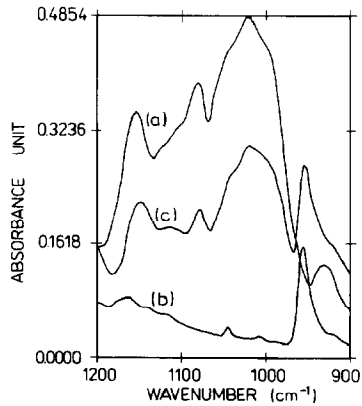

Fig. 9. FT-IR spectra in the 950–1200 cm^{-1} region of lyophilised samples for (a) pure 2% amylose, (b) pure 4×10^{-2} M Rose Bengal, and (c) 2% amylose in 4×10^{-2} M Rose Bengal ($X = 40$ Rose Bengal molecules per amylose molecule).

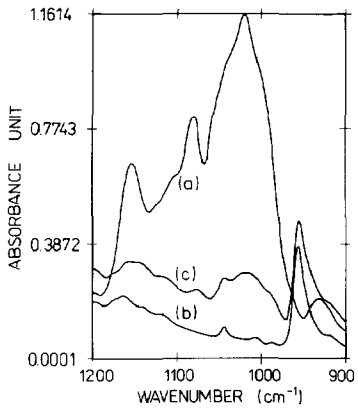

Fig. 10. FT-IR spectra in the 950–1200 cm^{-1} region of lyophilised samples for (a) pure 0.5% amylose, (b) pure 4×10^{-2} M Rose Bengal, and (c) 0.5% amylose in 4×10^{-2} M Rose Bengal ($X = 160$ Rose Bengal molecules per amylose molecule).

Bengal molecules, two effects were noticed: a decrease in the integral intensities of all bands and changes in the relative intensities of these bands.

The changes observed in the infrared spectrum of amylose–Rose Bengal samples have been considered separately for two models of amylose chain conformation in solution, namely, random coil and interrupted helix. We assumed that, in the interturn hydrogen bonding, stabilizing the helical structure, only internal OH groups at the C-2 and C-3 positions are involved. The external C-6 OH groups may be active in amylose–Rose Bengal complex formation and this process competes with helical hydrogen bonding. Amylose of dp 120 in the random coil form has 600 H atoms, at the C-1, C-2, C-3, C-4, and C-6 positions. (We did not take into account the hydrogen atoms at C-5 because these atoms are at the same carbon atom as the CH_2OH group. Because the CH_2OH group has a large degree of rotational freedom, it is much more probable that a Rose Bengal molecule will be attached to the CH_2OH and not to the hydrogen atom at C-5). Moreover, amylose in random coil form has 360 OH groups (at C-2, C-3, and C-6 positions) that may bind the appropriate Rose Bengal atoms. An amylose chain in the form of an interrupted helix has 360 hydrogen external atoms (at C-1, C-4, and C-6 positions) and 120 OH groups (at C-6 positions) which may interact with Rose Bengal. Hence, an amylose chain in the forms of random coil and interrupted helix has 960 and 480 atoms, respectively, which may be active in the complex-forming process.

Our FT-IR and Raman results indicate that a Rose Bengal molecule uses its oxygen and chlorine atoms, seven atoms in all, to combine with amylose. In Table II, we show the number of Rose Bengal molecules for each amylose molecule and the number of active atoms in a molecule of Rose Bengal compared with the number of active atoms in an amylose chain in both conformations, for the concentrations studied. The data in Table II indicate that, for the samples having a Rose Bengal concentration of 4×10^{-2} M and an amylose concentration lower

TABLE II

Number of active atoms in a Rose Bengal molecule per number of active atoms in amylose for the two models of the amylose chain conformation (coil and helix) [a]

Amylose (%)	Concentration (M)	X [b]	Y [c] (coil)	Z [d] (helix)
20	1.0×10^{-2}	4	28/960	28/480
10	5.0×10^{-3}	8	56/960	56/480
5	2.5×10^{-3}	16	112/960	112/480
2	1.0×10^{-3}	40	280/960	280/480
1	0.5×10^{-3}	80	560/960	560/480
0.5	2.5×10^{-4}	160	1120/960	1120/480

[a] $C_{RB} = 4\times10^{-2}$ M. [b] Number of Rose Bengal molecules per amylose molecule. [c,d] Number of active atoms in Rose Bengal per number of active atoms in one amylose chain, for both amylose models.

than 1%, all amylose molecules should be connected with Rose Bengal, for both conformations. Simultaneously for these samples, a decrease by ca. 88% of the intensities of all the bands in the 950–1200 cm^{-1} region has been observed. For a second sample of 2% amylose and 4×10^{-2} M Rose Bengal concentrations, we analysed the FT-IR spectra for the two models of amylose separately. If the random coil model is assumed, ca. 29% of the amylose molecules in a sample should be joined to the dye, whereas if the interrupted helix model is taken, 58% of these molecules should be joined. Our experimental FT-IR results for 2% amylose and 4×10^{-2} M Rose Bengal concentrations indicate a decrease by ca. 54% of the band intensities relative to those for pure amylose. This may indicate that amylose in water solution occurs in the form of an interrupted helix. A drastic decrease in the intensities of all the bands analysed suggests that amylose complexed with Rose Bengal forms a stiff network, in which amylose chains are joined through Rose Bengal molecules.

CONCLUSIONS

FT-IR and Raman spectroscopy results related to the Rose Bengal properties lead to the conclusions that (*1*) amylose molecules do not influence the Rose Bengal quinonoidal structure and (*2*) the oxygen and chlorine atoms of Rose Bengal are combined with amylose.

FT-IR spectra of lyophilised amylose alone and with Rose Bengal reveal some differences that are most pronounced in the region of CH and OH deformational vibrations. For high concentrations of Rose Bengal, a strong decrease of intensities of the bands corresponding to rotational vibrations were observed, which may indicate considerable limitation of CH and OH rotational vibrations of amylose groups bonded to Rose Bengal molecules. The dependence of the infrared signal of amylose in the structure-sensitive 950–1200 cm^{-1} region on the Rose Bengal concentration provides a convenient way to study the mechanism of complex formation. In this region, we observed a drastic decrease in the integral intensities

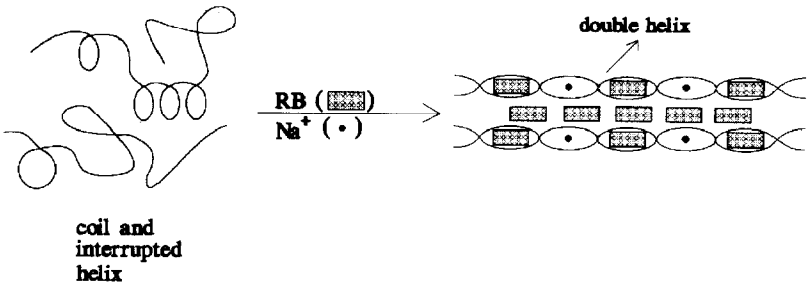

Fig. 11. Schematic model of amylose–Rose Bengal complex formation (the symbol RB denotes a Rose Bengal molecule).

of all amylose bands, induced by Rose Bengal. Therefore, we may conclude that a very stiff structure is formed, compared to that of the samples without the dye.

Simultaneously, the experimental results, obtained previously from fluorescence studies and by optical rotatory methods[21,28], indicate an increase in the content of the helix form of the amylose chain induced by Rose Bengal molecules. In Part I[28], we proposed two possible mechanisms of complex formation: helical inclusion (as indicated by UV–visible absorption and fluorescence results) and/or adsorption on the amylose chain. In this work, on the basis of the infrared investigation of amylose and the dye, we conclude that both of the earlier suggested mechanisms occur. Thus, for the amylose–Rose Bengal complex, we propose a model that involves amylose chain clusters or "domains" cross-linked through dye molecules, with other dye molecules located within amylose helices (Fig. 11).

Unfortunately, we could not determine the exact participation of the appropriate H and OH groups of amylose in the new structure; studies by another method, for example, by ^{1}H and ^{13}C NMR spectroscopy are required.

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