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Note

A spectroscopic study of amylose–Rose Bengal complexes

Wanda Maciejewska*, Krzysztof Polewski,
Department of Physics, Agricultural University, Wojska Polskiego 38/42, 60-637 Poznań (Poland)
and Monika Grundwald-Wyspiańska
Department of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań (Poland)

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Amylose interacts strongly with lipids, fatty acids, dyes, and many low molecular weight molecules[1-5], and forms helical inclusion (V-amylose) complexes with the guests accommodated within the amylose helix[6,7]. The complexes involve mainly hydrophobic interactions[8,9], and are stabilised by intramolecular hydrogen bonding[10]. The ability of amylose to form complexes is connected mainly with the conformation of the molecule and is correlated with the orientation of the functional groups in monomeric units[5,11-13]. In exploring the conformational properties of amylose and its interaction with dyes, we have studied, and now report on, amylose–Rose Bengal complexes in aqueous solutions as a model system.

The changes in the u.v.–visible absorption spectrum of 1.5×10^{-4}M Rose Bengal in the presence of various concentrations of amylose are shown in Fig. 1. The λ_{max} at 545 nm in the absence of amylose is shifted to 559 nm when 8% of amylose is present and the intensity decreases by ~ 25%. There is a linear dependence of the shift in frequency and the decrease in intensity only up to 4% of amylose. Above 4%, there is little effect on either parameter (saturation effect). Likewise, the shoulder at 507 nm is shifted to 520 nm when 8% of amylose is present, but the intensity does not change. Rose Bengal is an efficient photosensitiser and appropriate precautions are necessary. The absorption spectrum shows up an isosbestic point at 556 nm, which reflects the equilibrium between the dye in solution and that on the surface or within the amylose helix, where the environment is more hydrophobic and causes the shift of λ_{max} towards longer wavelength. The difference between the nominal concentration and that in the presence of amylose corresponds to the proportion of the dye in the complex, and the mono-anionic form of the dye is assumed to be responsible for all observed changes in absorption spectrum.

* Author for correspondence.

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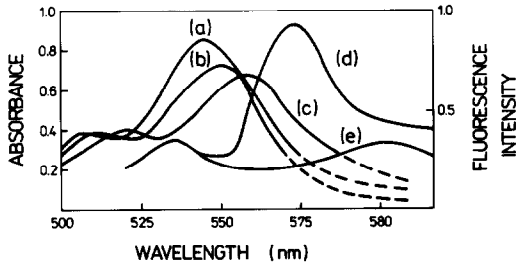

Fig. 1. Absorption (a–c) and fluorescence emission (d and e) spectra of 1.5×10^{-4}M Rose Bengal in aqueous solution (a and d), aqueous 2% amylose (b), and aqueous 5% amylose (c and e).

The fluorescence emission spectrum of an aqueous solution of Rose Bengal (Fig. 1) changes when amylose is added. Thus, the λ_{max} at 572 nm shifts gradually to longer wavelengths, *e.g.*, to 584 nm when 8% of amylose is present. Additionally, quenching of the fluorescence intensity occurs and this decrease is linear within the range of amylose concentrations used. The second, smaller peak with λ_{max} 534 nm does not change as the concentration of amylose increases and may simply reflect hydrogen bonding between Rose Bengal and water.

The F.t.-i.r. spectrum in the region 900–1500 cm^{-1} is sensitive to the structure of both amylose and Rose Bengal. Most bands reflect C–O and C–C vibrations[14,15], and assignment is difficult. Rose Bengal, in aqueous solution, has bands at 957 (sh), 1176 (sh), 1242, 1292 (sh), 1341, 1447, and 1487 (sh) (Fig. 2). The complex with amylose has a strong band at 1292 cm^{-1} and a weak band at 1242 cm^{-1}. These bands are due to symmetric stretching vibration of the C–C(= O) group[16]. It is conceivable that, when the Rose Bengal molecule binds to the amylose through the C=O bond, it effectively increases the mass of the oscillator and lowers the frequency. Changes in the relative

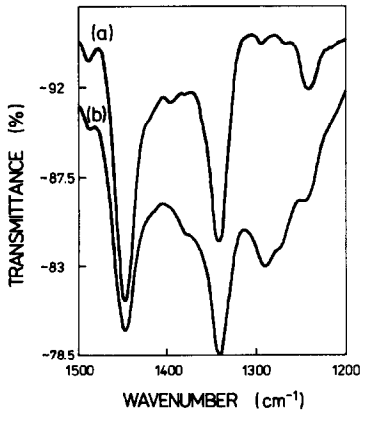

Fig. 2. F.t.-i.r. spectrum of 1×10^{-4}M Rose Bengal in aqueous solution (a) and in aqueous 5% amylose (b).

intensities were observed for the bands at 1341 and 1447 cm^{-1}, which are assigned to the C–O and COO^- groups in the dye.

The i.r. spectrum of an aqueous solution of amylose has bands at 1026 (C–OH bending), 1040 (sh) (C-1–H deformation), 1082 (C-1–H and C–OH bending), and 1154 cm^{-1} (C–O, C–C, and C–H deformation) (*cf.* refs. 17 and 18). When Rose Bengal is added to the amylose solution, a new peak appears at 1124 cm^{-1} (Fig. 3), which may be assigned to the related modes of C–H and C–OH deformation, and C–O–C antisymmetric stretching vibrations. Since the bands at 1026, 1082, and 1154 cm^{-1} do not change, the new peak at 1124 cm^{-1} may be connected mainly with the asymmetric stretching vibration of the C–O–C bond.

The F.t.-i.r. data suggest that formation of the amylose–Rose Bengal complex involves changes of the conformation of the amylose chain and confirmation was sought by a study of the optical rotation.

The value of $[\alpha]_{366}$ for amylose in the presence of Rose Bengal shows a low plateau region at a concentration of $> 1.5 \times 10^{-5}$M of the dye (Fig. 4). The ratio $([\alpha]_{366} - [\alpha']_{366})/$

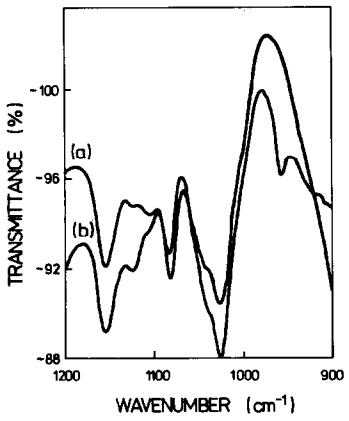

Fig. 3. F.t.-i.r. spectra of 5% amylose in aqueous solution (a) and in 1×10^{-4}M Rose Bengal (b).

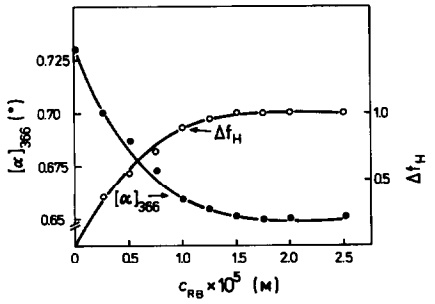

Fig. 4. Changes in the $[\alpha]_{366}$ of aqueous 0.2% amylose as a function of the concentration of Rose Bengal (c_{RB}); Δf_H is the relative change of the content of the helix fractions in the amylose chain.

($[\alpha]_{366} - [\alpha'']_{366}$), where $[\alpha]_{366}$ refers to an aqueous solution (+ 0.725°), $[\alpha'']_{366}$ refers to the plateau value (+ 0.65°) at a high concentration of Rose Bengal which may be taken[19] as an approximate linear measure of fractional helical content (f_H), and $[\alpha']_{366}$ refers to values between $[\alpha]$ and $[\alpha'']$, indicates the changes in the proportion of the helix fraction in the amylose chain (Δf_H) induced by Rose Bengal (Fig. 4).

It is concluded that amylose–Rose Bengal complexes may be formed by adsorption or by inclusion. The F.t.-i.r. data indicate significant participation of the C=O and COO^- groups in Rose Bengal in the formation of the complexes. The optical rotation data suggest that, for a concentration of Rose Bengal $> 1.5 \times 10^{-5}$M, the amylose chain adopts a stiff helical structure. Further investigations of the properties of amylose–Rose Bengal complexes are in progress.

EXPERIMENTAL

Preparation of amylose–Rose Bengal solutions. — Potato amylose (d.p. 100, as indicated by light-scattering) was obtained from Poland Chemical Reagents. The water was doubly distilled and deionised. Solutions prepared by digesting amylose powder for 20 min in boiling water were cooled to 25° at 12°/min and mixed with a solution of Rose Bengal to give the desired concentrations. For electronic spectroscopy and fluorescence measurements, the concentration of Rose Bengal (c_{RB}) was 1.5×10^{-4}M and that (c_a) of amylose was varied from 0.05 to 8%. For the F.t.-i.r. measurements, c_{RB} was 1×10^{-4}M and c_a was 5%. For the optical rotation measurements, c_a was 0.2% and c_{RB} was varied in the range $0.25–2.5 \times 10^{-5}$M. All measurements were carried out at room temperature.

Spectroscopy. — The absorption spectra were recorded with a SPECORD UV VIS spectrometer (Zeiss), using a 1-cm cell; the fluorescence spectra with a Perkin–Elmer MPF-66 fluorescence spectrophotometer; and the F.t.-i.r. spectra (950–1500 cm^{-1}) with a Bruker IFS 113v spectrometer [2 cm^{-1} resolution and a KRS5 6-μm cell].

Optical rotations. — A POLAMAT-A polarimeter was used with a 10-cm cell at 366 nm.

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