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# NMR AND COMPUTATIONAL COMPARATIVE STUDY OF THE AMYLOSE – BENGAL ROSE COMPLEXING IN DMSO SOLUTION

Abstract

Proton and carbon NMR spectroscopy was used to study the nature of amylose complexing with Rose Bengal in dimethylsulfoxide. Based on the analysis of chemical shifts changes in NMR spectra under the influence of dye-amylose chain interaction the computer molecular model of the helical amylose - Bengal Rose complex was proposed using the INSIGHT II and MOPAC programmes.

## Introduction

Molecular modeling of polysaccharides complexes in solution has been the subject of active research for many years [2-6, 8]. Many biopolymers are poorly water soluble and therefore, they have been studied in such organic solvents as dimethyl sulfoxide (DMSO), carbon tetrachloride, chloroform. Amylose, the linear starch component, with α-(1→4) - linked D-glucosyl units was one of them. It is well known, that DMSO is an effective amylose solvent and as the strong hydrogen bond acceptor may influence it. Especially, the chain configuration and flexibility are affected, on chemical and physical ways. The chain flexibility induces disordered or random coil states in solution.

It has been suggested that in the neutral aqueous solution amylose behaves as a "random coil" with short, loosely bound helical segments, whereas in DMSO the persistence of intramolecular hydrogen-bonding leads to an increase in the helical content and the compactness of the helical segment [1]. These changes in polymer behaviour due to a solvent affects the reactivity of amylose towards low-molecular compounds and on stability of the complex. Nevertheless there is a question whether dimethylsulfoxide is a good solvent for amylose complex formation because for the most known amylose-iodine complex this solvent suppressed the iodine binding [9].

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A complexing effect between amylose and heteronuclear photosensitizer, Bengal Rose in aqueous environment was shown previously [7, 10]. A simple model of the complex formation was proposed [10]. In this report, $^{1}$H- and $^{13}$C-NMR spectroscopy data were examined for amylose with Bengal Rose in DMSO-$d_6$ at different dye concentrations. The molecular model of six-fold amylose helix with associated Rose Bengal molecules in DMSO solution was considered.

**Materials and methods**

Commercial sample of potato amylose was a product of SIGMA, Bengal Rose (sodium salt) was purchased from ALDRICH Chem. Co. and deuterated solvents, $D_2O$ and DMSO-$d_6$, from I.B.J. Świerk/Otwock. Both solutions, of potato amylose and Bengal Rose (BR) in DMSO were blended at high temperature in appropriate proportions to obtain a desired dye concentration, from 5 to 20mM for 1% amylose. The measurements were performed after 24h storing at stable temperature. High resolution $^{1}$H- and $^{13}$C-NMR spectra were recorded with a Varian Unity 300 spectrometer operating at 300 MHz. The chemical shifts were measured with external 4,4-dimethyl-4-silapentane sodium sulfonate (DSS) in $^{1}$H-NMR spectra and external dioxane in $^{13}$C-NMR spectra. All the computer modeling study were conducted using the INSIGHT II and MOPAC programmes working in the SGI Iris Indigo 2 workstation.

**Results and discussion**

The effect of a solvent on the amylose - Bengal Rose (BR) complexing in solution was observed in the proton and carbon NMR spectra of both compounds in water and dimethylsulfoxide (Fig. 1). The H-NMR spectra of amylose in DMSO-$d_6$ exhibited all the resonances of hydroxyl protons [9], the signals for OH-2 and OH-3 strongly deshielded by intramolecular bonding and OH-6, which all disappeared after changing the solvent with $D_2O$ (Fig. 1c). The chemical shift displacement of all the single signals of the amylose proton resonance in the H-NMR spectrum in the presence of Rose Bengal molecules in DMSO-$d_6$ is presented in Table 1.

At lower BR concentration, only a small paramagnetic effect of 0.02 ppm for OH-6 hydroxyl group signal at $\delta = 4.58$ ppm in the amylose $^{1}$H-NMR spectrum could be observed. This insignificant effect points to lack of any drastic conformational changes in the amylose chain. The dye molecules did not disturb the intramolecular bonding with OH-3 and OH-2 hydroxyl groups in polymer. At low concentration they might cause some restrains for the freedom of hydroxymethylene groups. In the $^{1}$H-NMR spectrum of 1% amylose with a higher Bengal Rose concentration, 10 mM in DMSO-$d_6$ solution, strong deshielding of OH-2 ($\Delta\delta = 0.08$ ppm) and OH-3 ($\Delta\delta = 0.07$ ppm) signals was observed without change in the OH-6 resonance.

Table 1

Values of chemical shift for amylose proton signals in the $^1$H-NMR spectra of 1% amylose with Bengal Rose in DMSO-$d_6$ solution.

| Proton group | Chemical shift δ, ppm | | |
|---|---|---|---|
| | without RB | with RB of 5 mM | with RB of 10 mM |
| OH - 3 | 5.51 | 5.52 | 5.60 |
| OH - 2 | 5.40 | 5.41 | 5.50 |
| H - 1 | 5.10 | 5.10 | 5.11 |
| OH - 6 | 4.58 | 4.60 | 4.61 |

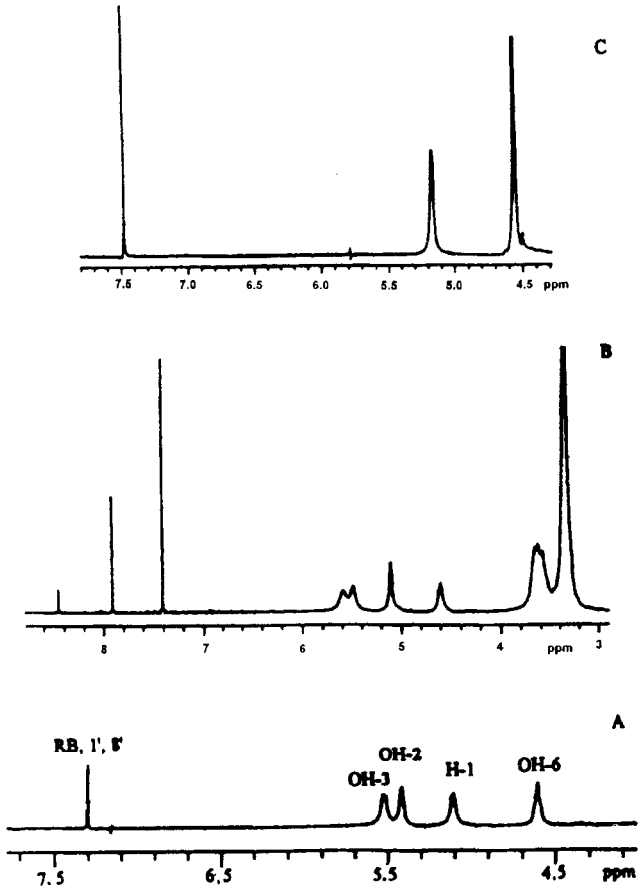


Fig. 1. $^1$H-NMR partial spectra of amylose ($c_{AM}$ = 1%) with Bengal Rose in DMSO-$d_6$ solution at 298K; BR concentration of 5 mM (A) and 10 mM (B) and in $D_2O$ solution with 20 mM BR concentration (C), at 300MHz.

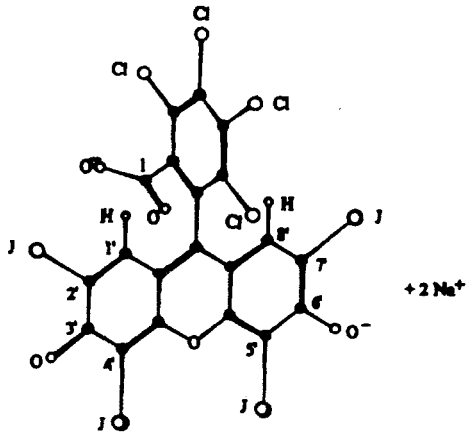

Fig. 2. Molecular structure of 4,5,6,7 - tetrachloro - 2',4',5',7' - tetraiodofluorescein (Bengal Rose).

Bengal Rose (Fig. 2), exhibited the single proton resonance at 7.40 ppm beyond the region ascribed to absorption of the polymer protons in the high resolution $^1$H-NMR spectrum (Fig. 1a) at lower concentration range studied. In the dye concentration of 10 mM this signal was resolved into three well separate signals at 8.46 ppm, 7.92 ppm and 7.41 ppm with the intensity ratio equal 0.12 : 0.4 : 1, respectively (Fig. 1b). It suggests a cooperative conformational effect in amylose chain forced by the Bengal Rose interaction, revealing an inhomogeneity of dye molecules state in the system. Taking into account the signal intensity ratio, equal to the ratio of absorbing protons in the proton NMR spectrum, one could obtain the degree of the assocciated BR molecules per the number of amylose monomer units. Among three NMR Bengal Rose signals observed, the most deshielded signal with the lowest longitudinal relaxation time indicated the most restriced dye molecules. The two other signals with a similar relaxation time might be involved in a cooperative dye interaction in DMSO. From the analysis of the signals the integration ratio suggested that two dye molecules were associated with six monomer units corresponding to the six-fold helical turn. There was no similar dye concentration effect on the $^1$H-NMR spectrum of amylose - Bengal Rose in the $D_2O$ solution. A considerable intensity decrease of the signals in the $^1$H-NMR spectrum of amylose in the presence of dye and their significant broadening pointed to a reduction of conformational mobility of the polymer due to the complex formation as well as to changes in the proton relaxation time of both compounds.

Nonequivalent dye subsystems were found in the amylose - RB complex in the DMSO-$d_6$ solution based on the proton NMR spectrum. They also changed the $^{13}$C-NMR spectra of both compounds. Effect of the cooperative dye-polymer interaction on

chemical shift displacements of the carbon signals in the system are presented in Fig. 3 and Fig 4. The assignement of the signals of BR and amylose carbon atoms was given in [5, 7]. At high Bengal Rose concentration the signal of the carbonyl group, C(1)OO, at $\delta$ = 164.3 ppm split into two signals, both deshielded as compared with the above, of 2.4 ppm and 0.6 ppm, respectively. It confirmed a multiphase dye state in the system. Other bands in the BR carbon NMR spectrum displaced very selectively. A considerable upfield displacement of the signals attributed to the Bengal Rose phenolic ring carbons in the region of 127 - 133 ppm might arise from the penetration of phenolic ring into amylose helix.

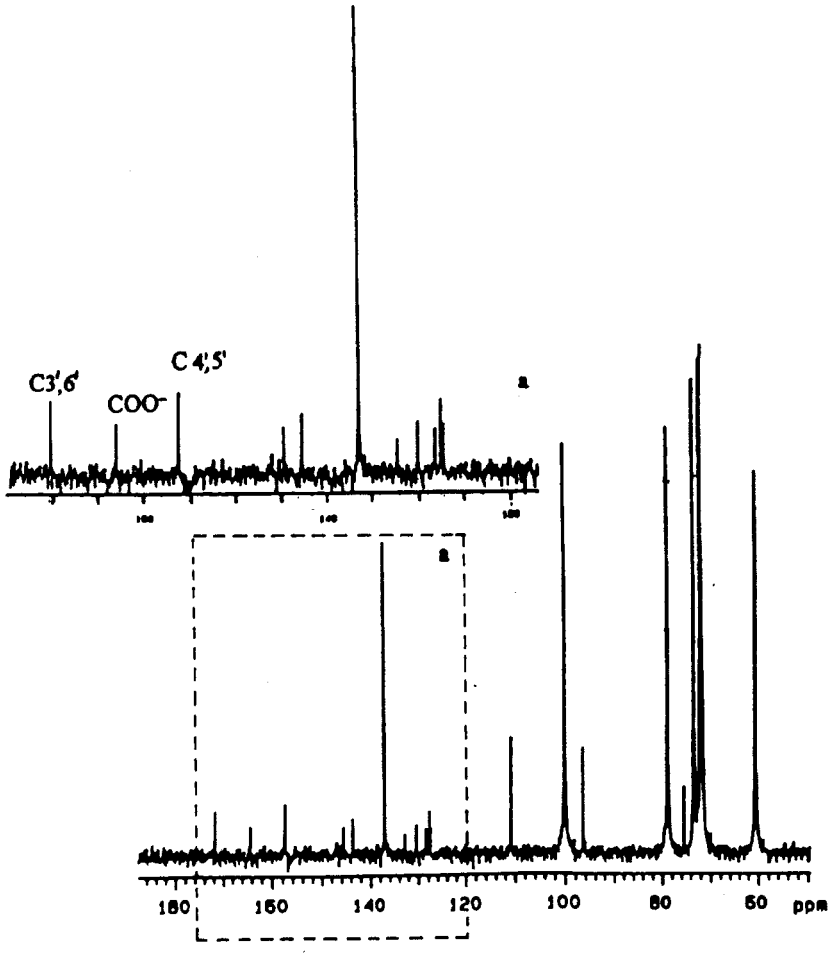


Fig. 3. $^{13}$C-NMR spectra of 1% amylose with Bengal Rose of 5 mM DMSO-$d_6$ solution at 295K.

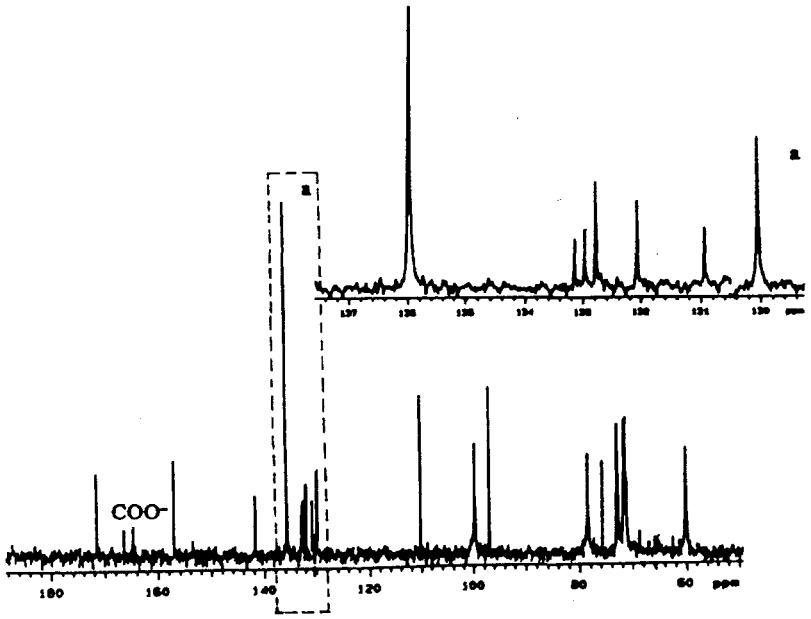


Fig. 4. $^{13}$C-NMR spectra of 1% amylose with Rose Bengal of 10 mM DMSO-$d_6$ solution at 295K.

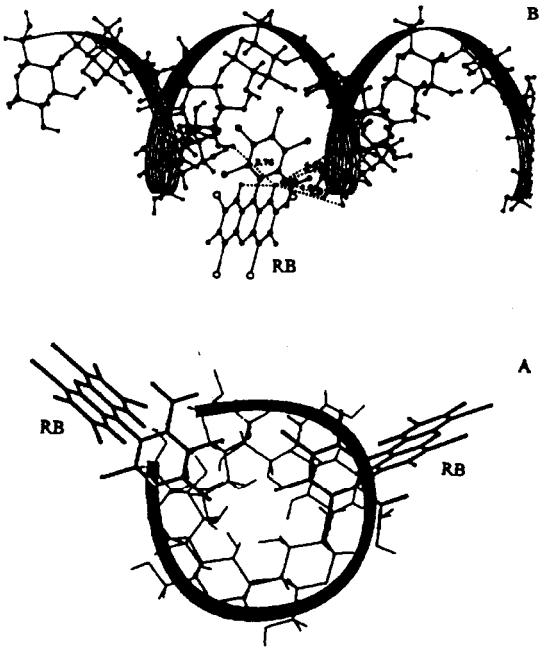


Fig. 5. Molecular model of six-fold amylose chain helix and Bengal Rose complexed in DMSO solution: projection along helical axis with two BR molecules approaching (A) and with one BR molecule (B).

Taking into account results from the $^{1}H$- and $^{13}C$-NMR analysis of amylose – Bengal Rose in DMSO-$d_6$ solution at different dye concentration, a molecular model of amylose helix - dye molecule complex could be proposed in Fig. 5. Bengal Rose molecule approaching the helical chain on the distance of 3 - 4 Å from the nearest polymer atoms, appropriate to hydrogen bonding, was confirmed with the computer simulation program.

## Conclusions

The $^{1}H$- and $^{13}C$-NMR study of the amylose - Bengal Rose complexing in deuterated dimethylsulfoxide at amylose concentration of 0.01 g/cm$^3$ and in 5 - 20 mM concentration range of BR showed a cooperative dye - polymer interaction at higher dye concentration. The considerable paramagnetic effect on OH-2 and OH-3 amylose proton signals and carbonyl BR signal splitting in the dublet accompanying the conformational polymer changes proved the role of these groups in conformational constraints. Based on the analysis of the NMR data a computational molecular model of single six-fold amylose helix was proposed with two associated dye molecules through phenolic ring approaching the helical chain.

The work was supported by KBN Grant 5 PO6 G 05408

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## MODELOWANIE KOMPLEKSU AMYLOZA - RÓŻ BENGALSKI W DMSO NA PODSTAWIE SPEKTROSKOPII NMR

Streszczenie

Zdolność kompleksowania amylozy z fotoczułym sensybilizatorem różem bengalskim w roztworze DMSO, jak wynika z badań metodami spektroskopii NMR, jest uwarunkowana stężeniem barwnika. Przy stężeniu powyżej 10mM w 1% roztworze amylozy pojawia się efekt przejścia konformacyjnego wymuszonego kooperatywnym oddziaływaniem barwnika. Podjęto próbę komputerowego modelowania kompleksu amyloza - róż bengalski w DMSO przy założeniu pojedynczej helisy i dwóch molekuł barwnika przypadających na sześcioczłonowy zwój.