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COMPUTER MODELLING OF AMYLOSE HYDRATION PROCESS IN THE PRESENCE OF BENGAL ROSE

Abstract

Simulation program HyperChem (Hypercube Inc., Waterloo, Canada) was used to study the conformational behavior of amylose helices in the presence of water and Bengal Rose. The structure was optimized with a program, which utilizes AMBER molecular force field calculations modified for carbohydrates. Single strand of amylose with the left-handed chirality has lower energy than the right-handed, double helix formed in antiparallel fashion from two left-handed strands. It has the lowest energy compared to other duplex conformation, interhelical stabilization occurs through hydrogen bonding O(2) — O(6) atoms. These results were available from computer calculations *in vacuo* and in the presence of water in the system. In water three hydration sites could be identified at O(3), O(6) and HO(6) for the amylose double helix. Bengal Rose when added to the system was located in the groove of double helix through the interactions of the xanthene structure to hydration sites of amylose, which might be deduced as a disruption of the water structure around the amylose helix.

Introduction

Amylose is a linear polymer formed by α -D-glucopyranose units linked through 1,4-bonds. As a main component of starch it is an important carrier of its physico-chemical properties. The experimental [1, 2] as well as modelled [3, 4] structure of the polysaccharide correlated with their physico-chemical and biological properties. Transition between crystallographical B-type and A-types were observed in dehydration/hydration cycles [5, 6]. Also NMR spectroscopic methods demonstrated the existence of different states of water in amylose. At higher humidities when more than two water molecules per glucose unit were available more mobile, less tightly bound water molecule appeared [7]. Geometry of the amylose double helix was widely discussed in the literature. On the basis of the X-ray fiber diffraction pattern data and computerized molecular modelling Wu and Sarko proposed a right-handed parallel-stranded double helix for both A and B amylose [2, 6]. Schulz et al. [4, 8] investigated

full conformation space of the double helix using potential energy minimization procedure within the framework of molecular mechanics. The results indicated the antiparallel left-handed helix. Experimental evidences exist [1, 3, 9], that environment like water influenced the conformational behavior of amylose. It was the starting point for recalculation of the amylose hydration process in the single and double helix in low dielectric and aqueous environment in the presence of Bengal Rose. The AMBER force field molecular mechanics [10] modified for carbohydrates was used.

Method

The molecular mechanic calculations were performed with the HyperChem (Hypercube Inc., Waterloo, Canada) simulation program. All energy study and the conformational behavior of amylose in water in the presence of Bengal Rose were studied with the Amber force field. The starting structure of amylose, the 16 α -D-glucopyranose oligomer, was built using the Sugar Builder program. The torsional angles Φ and Ψ at the glycosidic linkage were set at 2 and -17 as well as 20 and 5 degrees to get D-oligomer in a helical left or right-handed form.

The Bengal Rose geometry optimization and charges were calculated by the semiempirical PM3 method. During this procedure, the dye was assumed to be in an environment at pH 7. This implies that sodium salt was fully ionized to give Bengal Rose dianion.

Conformational Search option in the HyperChem program was used for the systematic conformational search on the glycosidic linkage. In this procedure Φ and Ψ torsion angles varied in 5° intervals within the whole angular range.

Docking simulation between left and right handed oligosaccharides and with Bengal Rose on the amylose duplex were performed using the Periodic Box option with TIP-3 water molecules. These calculations were conducted with global helical parameters maintained constant.

Results and discussion

Results of the molecular calculation for single and double left and right handed amylose helices, their parallel and antiparallely stranded conformations *in vacuo* and in water are collected in Table 1. It can be noted that a single strand with left-handed chirality had lower energy than that right-handed. As proved by the HYDROGEN BOND Option in the HyperChem program no hydrogen bonding in single helix existed. In case of amylose double helix the lowest energy double helix was formed of two left-handed parallel-stranded conformation and the interhelical stabilization occurred through hydrogen bonding between O(2) and O(6) atoms. When the calculations

are performed in aqueous environment, i.e. in the presence of Periodic Box Option with TIP-3 water molecules the total energy in all cases changed. The calculated energies for right-handed double helices (parallel stranded or antiparallel stranded) had higher energetic minima than their left-handed analogues. Left-handed helices were energetically favorable and differences between them were within 5kJ per unit. It could be noticed that in aqueous solution the hydration pattern could be identified by the single and double helices. The conformation energy in water was minimized under the condition of rigid global helical parameters. This simplification might be justified by much smaller relaxation times for the reorientation of water molecules in comparisons with groups of atoms of the macromolecule. Such behavior regarding hydration of amylose was expected and supported by X-ray diffraction studies [11]. Amylose double helix was supported by periodical formation of intermolecular hydrogen bonds between hydroxyl groups and oxygen atoms and by an arrangement of hydrophobic contacts in the core of the double helix. The hydrogen bonding between appropriate hydroxyl group and oxygen was also evidenced by our calculations. It is also known that an increasing amount of water causes transition from the A-type to B-type conformation of amylose [2, 5]. The hydration pattern displayed by the amylose duplex

Table 1

Total potential energy (kcal) of the amylose and amylose + water systems and in the presence of Bengal Rose (BR). In the double helix data first number is given for parallel and second for anti-parallel strand. A_R and A_L - right-handed and left-handed amylose strand, respectively.

	A_L	A_R	A_L-A_L	A_R-A_R	A_L-A_R
in vacuo	-242	-281	-573 -577	-486 -477	228 266
water	-134	-148	-1019 -994	-921 -893	147 131
water+BR	22	32	-171 -125	-105 -101	725 668

is given in Figure 1. Three identified hydration sites correspond to the polar O3, O6 atoms and the HO6 group. Moreover, a number of water bridges was observed. The O2, HO3, O3 and HO2 moieties were involved in the intrahelical hydrogen bonds and they were less accessible to water molecules. The hydration sites were not energetically equivalent. The O6 group was well exposed to the environment and accounted for the majority of the hydrogen bonds with water. Some competition between O3 and O2 atoms appeared decreasing the hydration in that area of the amylose unit. Thus, all polar groups (except the glycosidic O1 atom) are involved in the hydrogen bonds with water or within the double helix. Thus, a single glucose unit accepted 3 water molecules forming a regular hydration shell around the double helix.

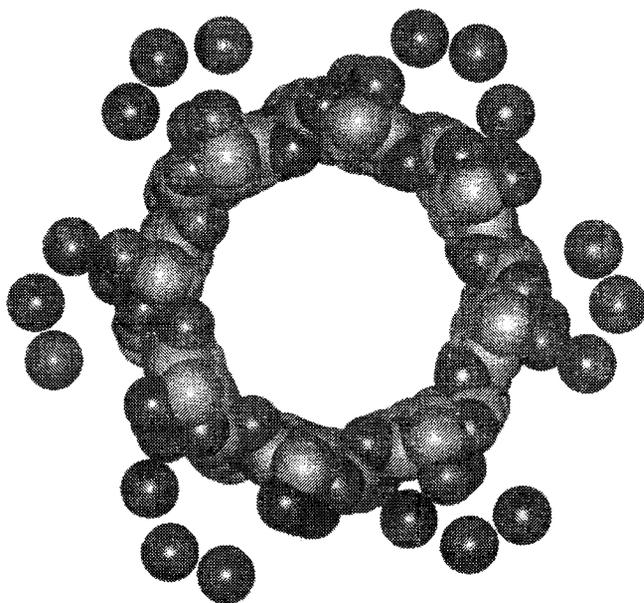


Fig. 1. Segment of the left-handed antiparallel-stranded double helical amylose with the corresponding 3 hydration sites per glucose residue.. The top view of double helix segment of six glucose residues per strand.

When Bengal Rose was added to the amylose double helix system in water the dye located in the groove of double helix through interactions with the xanthene structure as found in docking studies. It was also obvious that the presence of Bengal Rose ruined the water structure around the amylose helix. It is evident from Table 1 the total potential energy of the amylose system in water in the presence of dye was much higher because the presence of the dye disturbed the water-water interactions. Diameter of the amylose helix was large enough to adopt Bengal Rose inside of the amylose helix. However, some hydrophobic interactions inside the helix might preclude such location. It is clear from our previous studies [12, 13] that the presence of Bengal Rose influenced the amylose helix. We suggest that Bengal Rose might interact with amylose either by an intercalation process, adsorption process or the other non-covalent bonding and these calculations seem to confirm our suggestion.

Reported calculation and discussion presented in this paper indicate that the left-handed double helix strand of amylose should be a well soluble species. Because the solubility of amylose can be achieved by boiling at diluted solution we may expect that the complex around 90°C should be either single stranded or in the double helical form

antiparallely stranded and left-handed. In contrast, amylose in nature is insoluble and serves as a glucose storage what indicates that in native state the amylose conformation should be different to provide it to be water insoluble.

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MODELOWANIE KOMPUTEROWE PROCESU HYDRATACJI AMYLOZY

Streszczenie

Do zbadania stanów konformacyjnych helisy amylozy w obecności wody i różu bengalskiego zastosowano program do modelowania komputerowego HyperChem (Hypercube Inc., Waterloo Kanada). Badane struktury optymalizowano przy pomocy programu AMBER, wykorzystującego metody mechaniki molekularnej zmodyfikowanej dla węglowodanów. Pojedyncza lewoskrętna spirala amylozy ma niższą energię niż prawoskrętna, podwójna antyrównoległa struktura złożona z dwóch lewoskrętnych spiral posiada najniższą energię w porównaniu z innymi konformacjami dupleksów, a interhelikalna stabilizacja zachodzi poprzez wiązanie wodorowe między atomami O(2) a O(6). Zbliżone wyniki podane powyżej otrzymano zarówno dla obliczeń *in vacuo*, jak i w obecności wody w układzie. W obecności wody znaleziono trzy miejsca na powierzchni helisy, gdzie następuje hydratacja, i są to miejsca przy atomach O(3), O(6) i HO(6). Doadanie barwnika, różu bengalskiego, do amylozy lokuje go w bruzdzie spirali poprzez oddziaływanie struktury ksantenowej z miejscami hydratacji, co może być opisane jako zaburzenie struktury wody wokół helisy amylozy. ☒