# Chemistry of Natural Compounds and Bioorganic Chemistry

## Conformations of polysaccharides in solution, gels, and crystals

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"Solution—gel—crystal" phase transitions in a polysaccharide—water—salt system were examined. Polysaccharide helices were shown to exhibit identical conformation in solutions, gels, and crystals containing up to 50 % of water.

**Key words:** polysaccharide; solution; gel; crystal; conformation; helix.

In the crystalline state, polysaccharides exist as extended helices stabilized by inter- and intramolecular hydrogen bonds.[1—5] According to literature data,[4,6,7] the conformation of these helices depends on the humidity of the crystalline specimens. In this case, water is not an inert solvent, but is a complex-forming agent.

Studies of the conformational dynamics of polysaccharides by X-ray diffraction in the solid-phase polysaccharide—water—salt system show that dehydration of the crystals of the polymer-salt complexes is accompanied by gradual extension of the polysaccharide helices.[8—10] Thus, one can assume that the humidification of such specimens would induce the reverse processes stipulated by the contraction of the polysaccharide helices (crystal—gel—solution transition).

The extension of polysaccharide helices upon dehydration of the complexes was monitored by following the increase in the length of the translational vector of the crystal lattice, which characterizes the period of identity along the axis of the macromolecule,[8—10] and their optimal conformations (the most preferable energetically) for each value of this vector were computed by the molecular mechanics method.[11] As a result of comparing the energetic parameters of conformers with various periods of identity, it was established that the values of the minima of potential energy of helices remain practically constant with extension, *i.e.*, the conformational transitions of polysaccharides in the solid phase are isoenergetic and are caused by a change in entropy.[8,9,12] The range of experimental values of periods of identity in which polysaccharides retain the minimum values of potential energy may be called "the range of isoenergeticity".

However, computations for extremely contracted (solution, gel) and totally extended (dehydrated crystal) polysaccharide helices show that this range can theoretically be expanded substantially. Hence, the dependence of the potential energy of the polysaccharide helix on its period of identity is characterized by the presence of a broad minimum. Obviously, outside these limits, the probability of the existence of helical conformers for any polysaccharide is practically negligible.

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Thus, the range of isoenergeticity of any chemically regular polysaccharide characterizes in this case the most probable area of existence of all its helical conformers in the liquid, gel-like, and solid phases.

The transition from the crystalline state into the liquid state as the content of water in specimens is increased, is accompanied by the cleavage of intermolecular hydrogen bonds and gradual contraction of the polysaccharide helices, which results in the retention of only intramolecular hydrogen bonds in these helices. The following experiment suggests the existence of helices stabilized by intramolecular hydrogen bonds in solution.[13] An aqueous solution of high-molecular laminaran (1,3-β-D-linked glucan) exists as a viscous-flow gel; its $^{13}C$ NMR spectrum displays very broad lines. After addition of alkali, the lines begin to narrow gradually, and in the range of concentrations from 0 to $C_N$ ($C_N$ = 0.2 mol $L^{-1}$), their positions in the spectra are absolutely identical. At concentration $C_N$, an abrupt lowfield shift of the C(1), C(3), and C(4) signals is observed, and a subsequent increase in the concentration of alkali does not result in any spectral changes.

The results obtained are explained as follows.[13] The presence of the broad lines in the spectra of laminaran gel is due to the low conformational lability of the polysaccharide helices associated with each other due to intermolecular hydrogen bonds. The addition of alkali in the concentration range $0-C_N$, results in the cleavage of these bonds, which causes an increase in the conformational lability of the helices, and, hence, line narrowing in the NMR spectra. The cleavage of the intermolecular bonds does not affect the structure of the laminaran helices, so the positions of the lines in the NMR spectra in the alkali concentration range $0-C_N$ remain constant. At concentration $C_N$, when the alkali directly affects the intramolecular hydrogen bonds, the conformations of the helices change, which results in the lowfield NMR shift of the "bridged" C(1) and C(3) atoms and of the C(4) signal, the hydroxyl group at which is coordinated by a hydrogen bond with the O(5) atom of the neighboring monosaccharide residue in native laminaran.

Thus, the abrupt lowfield shift of the C(1), C(2), and C(4) signals (by 2.8, 3.2, and 0.9 ppm, respectively) is explained[13] by the cleavage of the intramolecular O(5)...HO(4) hydrogen bonds that stabilize the helical conformation of laminaran in an aqueous solution. From the values of these shifts, one can conclude that the conformation of the macromolecule changes insignificantly. At the same time, the loss of conformational rigidity due to the disappearance of the O(5)...HO(4) bonds results in folding of the polysaccharide helices caused by fractures in the "backbone" in the helicized coils consisting of short, helical fragments.

On the basis of the experimental data, it was concluded[13] that in solution and in gel the conformation of the laminaran chain is basically the same. This chain is a helix, whose translational regularity is violated due to the fractures of the helical backbone in contrast to the helix observed in the crystalline state. Later, this conclusion was confirmed by solid-phase NMR spectroscopy.[14]

In the above experiment,[13] native, high-molecular-weight laminaran was used to determine the identity of the microstructure in solution and in gel. In water devoid of alkaline additives, it always exists in a "gel-like" state. Evidently, to solve the problem, the authors[13] could use only one method, *viz.*, the gradual destruction of the gel with alkali, which can cleave the intermolecular and the intramolecular hydrogen bonds characteristic of any polysaccharide gel. However, along with the action on the hydrogen bonds, alkali also results in the ionization of the carbohydrate hydroxyls, the volume of which increases strikingly due to the appearance of a negative charge at the oxygen atoms; this, in principle can influence the conformation of the helix.

Therefore, the small conformational changes observed in the $^{13}C$ NMR spectra upon the addition of alkali to the gel may have two causes: cleavage of the O(5)...HO(4) intramolecular hydrogen bonds and ionization of the hydroxyl groups.[13]

In order to exclude the influence of alkali on the conformation of polysaccharides during the change in the aggregate state of the polysaccharide—water system, we studied the process of gel formation ("solution—gel" phase transition), which proceeds during the addition of alkali-metal salts inert with respect to the hydroxyls and hydrogen bonds to the solution, rather than the process of gel destruction ("gel—solution" phase transition) under the action of KOH or NaOH accompanied by the folding of the helices into coils. Gel formation in neutral solutions of polysaccharides in water-salt systems is the result of the unfolding of coils into helices and the subsequent association of these helices into macromolecular aggregates.[15,16]

In the present work, two easily water-soluble, low-molecular weight polysaccharides (mol. wt. *ca.* 4000—5000), *viz.*, linear dextran and laminaran, served as the objects of the study of the "solution—gel" phase transitions. An increase in the salt concentration in an aqueous solution (the polymer : salt mass ratios varied from 1 : 1 to 1 : 10) caused gradual broadening of the lines without changing their initial positions in the $^{13}C$ NMR spectra. This indicates that the process of chain unfolding followed by chain association in macromolecular aggregates proceeds with the retention of the initial conformation of the polysaccharides.

It is known that in gels prepared by the addition of alkaline-metal salts to aqueous solutions of polysaccharides, the polymer chains exist as slightly extended helices.[15,16] Hence, based on the identity of the NMR spectra of liquid and viscous-flow specimens, one can conclude that folded polysaccharide chains in solutions are not statistical, but are helicized coils having the conformation of helical fragments similar to that of helices in gels.

Thus, the study of the processes of dissociation (gel—solution) and association (solution—gel) of macromolecular carbohydrates under the action of alkali and salt indicates that the reversible helix—coil and coil—helix transitions are accompanied mainly by a change in the shape, but not in the conformation, of the helices. Small changes in the $^{13}C$ NMR spectra in the first case and their absence in the second arise from the fact that an alkali, in contrast to a salt, affects both the shape of the helix and, partially, of some of its fragments due to cleavage of the intrachain hydrogen bonds and the appearance of ionized hydroxy groups, which are more bulky than neutral hydroxy groups.

According to the $^{13}C$ NMR spectroscopy and X-ray analysis data,[17—19] disaccharides in solution and in the crystalline state not only have very similar conformations, but have the same intramolecular bonds. However, this is not the case for polysaccharides, since their conformation depends substantially on the content of water in the specimens under study.[4,6,7]. At the same time, it is known that the amount of water bound to a polysaccharide usually does not exceed three molecules per monosaccaride residue even in solution.[20] Thus, one can assume that in solution, in gel, and in a crystal with a high content of water (40—50 %), the fragments of polysaccharide helices should possess similar conformations.

The identity of helical conformers in gels and humid crystals in the polysaccharide—water—salt system is confirmed by the following experiment. During the slow dehydration of gels containing 50 % of water, an intense, diffuse halo characteristic of the amorphous phase is first observed in diffractograms, and on this background, narrow, well-resolved crystallographic reflections appear. During the further dehydration of the specimen, the amorphous halo gradually disappears, accompanied by continuous changes in the diffractograms.

The presence of the amorphous halo in the diffractograms indicates the existence of a gel-like phase, and the appearance of narrow crystallographic reflections on its background shows the formation of the first crystalline modification in this phase, the content of water in which is approximately equal to the content of water in the gel. The gradual disappearance of the amorphous halo is related to the transition of the gel-like phase into the crystalline phase, and the gradual change in the diffractograms is associated with the rearrangement of the crystal lattice of the complexes due to the isoenergetic conformational transitions caused by the longitudinal extension of the polysaccharide helices.[8—10,12]

As can be seen from the above experimental data, the changes in the conformations of the helices during the dehydration of polysaccharide—salt complexes begins in fact after the disappearance in the specimen of the first; most humid crystalline modification formed at the initial stage of the gel—crystal phase transition. Hence, one can conclude that the polysaccharide helices in gels and humid crystals containing 50 % of water possess similar conformations.

Studying the humid crystalline specimens by X-ray diffraction is the easiest way to determine the conformation of helices existing in liquid as well as the gel-like and solid phases. However, diffractograms of such specimens are not always interpretable due to the presence of an extremely small number of reflections. In this case, another approach can be used to solve the problem. It is known[8—12] that during dehydration of crystalline specimens, as the polysaccharide helices undergo isoenergetic extension, the quality of the diffractograms improves due to a decrease in the number of defects in the packing of the macromolecule, and the number of reflections increases. Therefore, the diffractograms of dry specimens, as a rule, are more informative than those of humid specimens.[12] It should also be noted that during the isoenergetic extension of polysaccharide chains in the crystal, not only the potential energy of the helix, but also its symmetry remain constant.[8—10,12] Hence, having determined the period of identity of a given compound from the diffractogram of a dry specimen and having calculated for this period the symmetry, the pitch, the minimum of potential energy, and the conformational parameters of the helix,[8—10] it is not difficult to ultimately create a model of the most probable spatial structure of the chains of the polysaccharide under study in solution, in gel, and in the crystal with a high content of water by compressing the helix to the lower border of "the range of isoenergeticity", *i.e.*, by decreasing its pitch.

Thus, the "solution—gel—crystal" phase transitions observed by NMR spectroscopy and X-ray diffraction in the course of slow evaporation of solutions of polysaccharides in water-salt systems and dehydration of humid crystals occur in two steps. At the first step (the "solution—gel" transition), unfolding of the coils of the polysaccharide helices with the retention of their initial conformations (the helical microstructure) takes place, which is confirmed by the identity of the NMR spectra of the solution and the gel. At the second step (the "gel—crystal" transition), a change in the conformation of the helices with the retention of their initial symmetry caused by the elongation of the polysaccharide chains is observed. This follows from the smooth rearrangement of the crystal lattice of the polymer—salt complexes accompanied by a gradual increase in the period of identity of the macromolecules.

The processes that proceed in solution and in the crystalline state related to the change in the shape (solution) and the conformation (crystal) of the helices, are isoenergetic. Therefore, the range of the existence of helical conformers with different degrees of extension and with the same symmetry can be described by the "range of isoenergeticity".

Therefore, the following procedures are needed to determine the conformation of the helix of any chemically regular polysaccharide in the liquid, gel-like, and

crystalline phases with a high content of water: 1) use X-ray diffraction data to compute by the molecular mechanics method the main geometric characteristics of the helix existing in a dry crystal; 2) vary the length of the repeating polysaccharide unit to plot the dependence of the potential energy of this helix on its period of identity; 3) determine the borders of the planar area of the above dependence, which characterizes the range of isoenergeticity, within the limits of which lies the area of the existence of all helical conformers of the polysaccharide under study; 4) calculate the spatial structure of the helical conformers possessing periods of identity lying in the lower part of this range of isoenergeticity.

The slightly extended helices (with small periods of identity) thus found represent a set of conformers that are formed in solutions, gels, and humid crystals.

## Experimental

In the polysaccharide—water—salt system (P—W—S, sodium acetate as the salt component), the "solution—gel" phase transition (S—G) was performed by increasing the concentration of salt in the solution (the polysaccharide : salt mass ratios (P : S) were increased from 1 : 1 to 1 : 10) and concentrating the solution (P : S = 1 : 2); the "gel—crystal" phase transition (G—C) was carried out by slow dehydration of the gel (P : S = 1 : 2).

The S—G phase transition was studied by liquid-phase NMR spectroscopy, and the G—C phase transition and the process of dehydration of crystals were studied by X-ray diffraction and molecular mechanics.[8—12]

The $^{1}H$ and $^{13}C$ NMR spectra were recorded with a Bruker AM-300 instrument at 80 °C using methanol as the internal standard (δ 50.14 from $Me_4Si$).

The diffractograms were obtained with a DRON-3 diffractometer (Kα radiation, the Kβ component was absorbed by a Ni filter). The rate of the pulse counter was 0.5 deg $min^{-1}$; the chart speed was 60 mm $h^{-1}$.

The diffractograms of crystalline modifications arising upon dehydration of the P—W—S complexes were interpreted by the Ito method.[21]

The energetic and conformational parameters of the polysaccharide helices of the corresponding modifications were evaluated from the X-ray diffraction data using the method of molecular mechanics.[8—12]

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