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Small-Angle X-Ray Scattering from Heparin in Solution

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Introduction

Heparin is one of the most important glycosaminoglycans (mucopolysaccharides) because of its anticoagulant activity. Chemical degradation studies of heparin show that heparin is an alternative copolymer consisting of α-D-glucosamine and uronic acids, either β-D-glucuronic acid or α-L-iduronic acid, joined together with 1→4 glycosidic linkages. X-ray diffraction studies on well-oriented fibers of heparin (ATKINS and NIEDUZYNSKI, 1977) suggested that the conformation of heparin is a 2_1 helix with a disaccharide as a structural unit. STONE (1967) suggested, from ORD studies on heparin solutions, that heparin has an ordered structure similarly to helical polypeptides.

STIVALA et al. (1968) measured the X-ray small-angle scattering from heparin in solution, and suggested that the conformation of heparin is a random coil. Their experiment, however, was limited to a relatively small angular region. We measured the scattering at relatively large angles, which reflects the microstructure of the molecule, and compared it with the theoretical curves based on various molecular models of heparin.

Models and Calculation

Since the primary structure of heparin is not definitely known, and for simplicity of calculation, we confined our models to alternative copolymers consisting of only two kinds of saccharides, one of which was α-D-glucosamine and the other was either β-D-glucuronic acid or α-L-iduronate. The dominant conformations of α-D-glucosamine and β-D-glucuronic acid are regarded as the $^4C_1$ conformation. On the other hand, α-L-iduronate may exist both in the $^4C_1$ and $^1C_4$ conformations, because both conformations of this saccharide are almost equally energetically stable. Therefore we adopted the three models listed in Table 1.

The positions of the atoms in each chair conformation was fixed according to the standard coordinates determined by ARNOTT and WINTER (1972) from various crystallographic data of saccharides. The positions of the atoms in the side groups were fixed according to the initial conformations proposed by ATKINS and NIEDUZYNSKI (1977) for the analysis of the X-ray fiber diffraction from stretched heparin.

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TABLE 1

The models used for the calculation of scattering functions. All of them are alternative copolymers in the form of $-(AB)_n-$.

model	A	B
1	α-D-glucosamine ($^4C_1$)	β-D-glucuronic acid ($^4C_1$)
2	α-D-glucosamine ($^4C_1$)	α-L-iduronate ($^4C_1$)
3	α-D-glucosamine ($^4C_1$)	α-L-iduronate ($^1C_4$)

We assumed regular conformations of the main chain, which are described by the four rotational angles ϕ_{AB}, ψ_{AB}, ϕ_{BA}, ψ_{BA} defined in Fig. 1. The rotational angles seem to be unable to take arbitary values because of the steric hendrance due to the bulkiness of the saccharides in chair conformations.

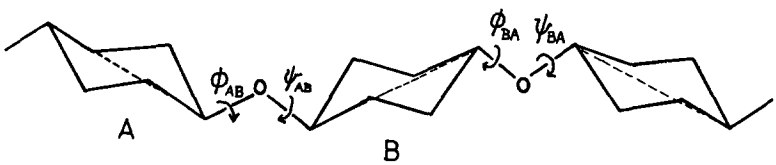

Fig. 1. The schematic representation of the main chain of the models, and the definition of the rotational angles. The angles are measured relative to the cis conformation and taken as positive for a right-handed rotation.

We calculated the allowed regions for the rotational angles according to RAMACHANDRAN et al. (1963), who assumed the hard sphere potencials between nonbonded atoms. Fig. 2 shows the results of the calculation for models 1 and 3. We took into consideration only the effects of the pyranose rings and the atoms bonded directly to them. The calculation was made at intervals of 10°. These figures indicate that the rotation around the C-O and O-C bonds is limited in rather small regions, which agrees with the result obtained by NAGARAJAN and RAO (1979).

Once the model and the four rotational angles are fixed, the positions of all the atoms in the molecule are determined, and therefore the scattering function can be calculated. The scattering function P(h) is given by the well-known equation (1).

$$P(h) = \frac{1}{N^2} \sum_{i=1}^{n} \sum_{j=1}^{n} f_i f_j \frac{\sin hr_{ij}}{hr_{ij}} \qquad (1)$$

where N is the total number of electrons in the molecule, and $h=(4\pi/\lambda)\sin\theta$, where 2θ is the scattering angle, and λ the wave-

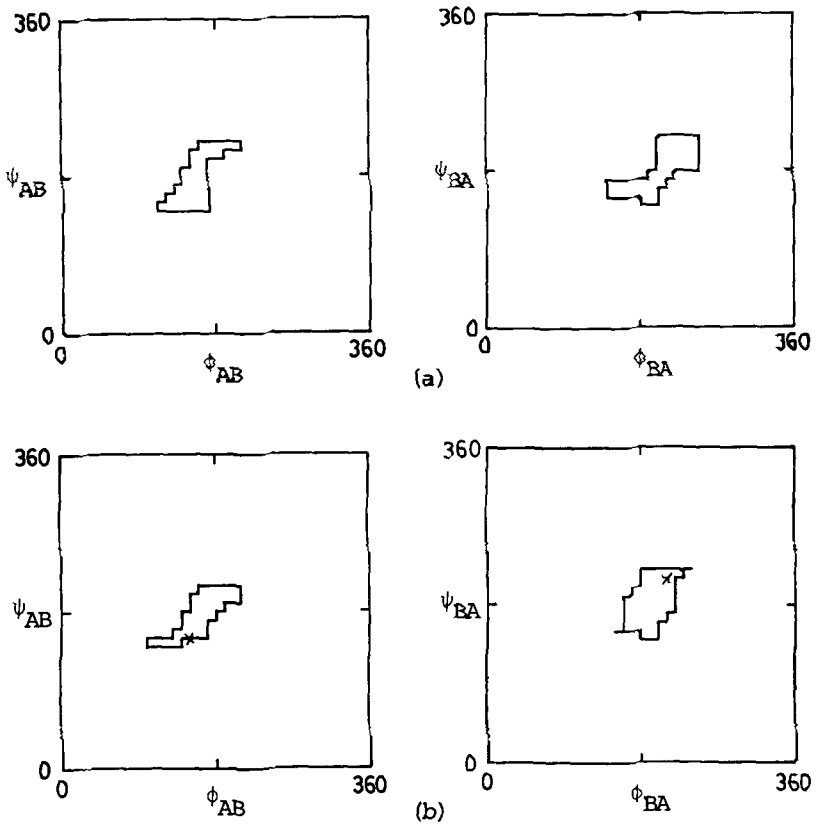

Fig. 2. The allowed regions of the rotational angles (inside of the contour) for model 1 (a) and model 3 (b).

length of X-ray. f_i and f_j are the scattering factors of the i-th and the j-th atoms, respectively.

By introducing the distribution function $H(r_k)$ defined by the sum of the products of the scattering factors of the atom pairs located at the distance r_k, we can rewrite eq. (1) in the form of the single summation as eq. (2).

$$P(h) = \frac{1}{N^2} \sum_k H(r_k) \frac{\sin hr_k}{hr_k} \qquad (2)$$

The application of the hypothetical bonds defined in Fig. 1 (REES, 1969; SUGETA and MIYAZAWA, 1967) simplifies the transformation matrices and therefore the calculation of $H(r_k)$. We regarded the atomic number as the scattering factor, because in the angular range considered here the angular dependence of the scattering factor does not appreciably affect the shape of the scattering

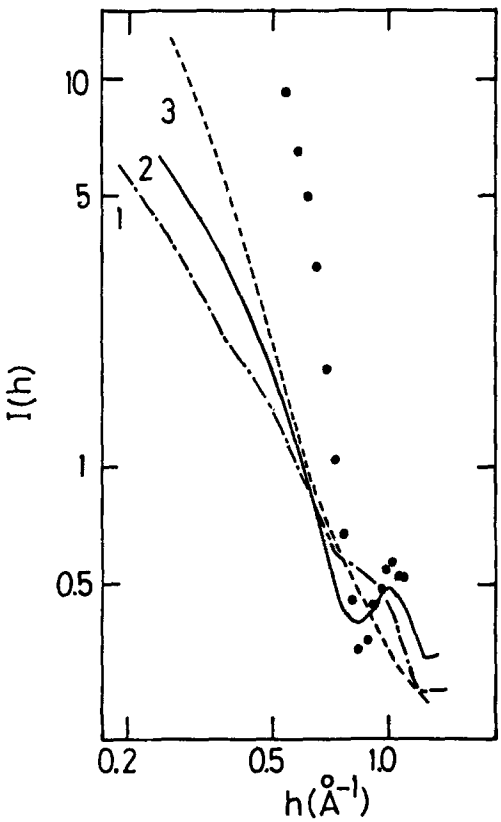

Fig. 3. The log-log plot of the scattering curves for heparin. The filled circles show the experimental scattered intensity. Curve 1 is calculated for model 2 with (ϕ_{AB}, ψ_{AB}, ϕ_{BA}, ψ_{BA})=(150°, 150°, 250°, 190°), Curve 2 for model 3 with (ϕ_{AB}, ψ_{AB}, ϕ_{BA}, ψ_{BA})=(150°, 150°, 210°,210°), and Curve 3 for model 3 without restrictions for ∠COC angles and the rotational angles.

function of the molecule (BRAM and BEEMAN, 1971). The parameters of helices, such as the pitch, were determined according to the method of SUGETA and MIYAZAWA (1967).

Experimental

Heparin was purchased from Nakarai Chemicals, Ltd., and used without further purification. The solvent was twice distilled water, the electronic conductivity of which was less than 1 μmho. X-ray small-angle scattering was measured with a Kratky camera in an angular range of 2θ=8-16° at the temperature of 25°C.

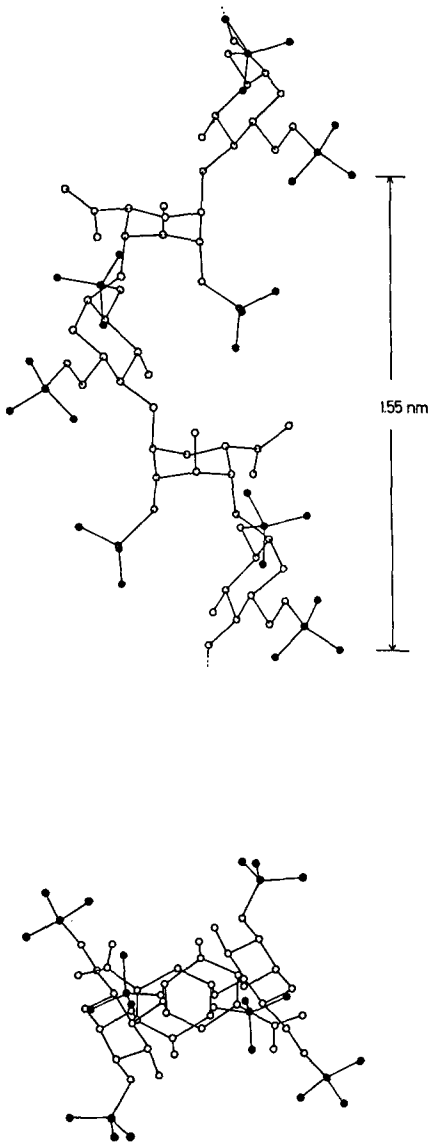

Fig. 4. The conformation of heparin model 3 corresponding to Curve 2 in Fig. 2.

Result and Discussion

The experimental scattered intensity is plotted by the filled circles in Fig. 3. A peak appeared around h=0.95 $Å^{-1}$. Taking into consideration the result of ATKINS and NIEDUZYNSKI (1977), we calculated the scattering functions for 2_1 helices with various combinations of the rotational angles.

Only a shoulder appeared in the theoretical curves for models 1 and 2, whereas an appreciable peak appeared in the curves for model 3. Curve 1 in Fig. 3 shows an example of the scattering curves for model 2 with (ϕ_{AB}, ψ_{AB}, ϕ_{BA}, ψ_{BA})=(150°, 150°, 250°, 190°), and Curve 2 with (ϕ_{AB}, ψ_{AB}, ϕ_{BA}, ψ_{BA})=(150°, 150°, 210°, 210°). The latter combination of the angles is shown by x in Fig. 2(b), and the corresponding conformation, the pitch of which is 0.775 nm, is drawn in Fig. 4. Curve 3 is also based on model 3, but on a hypothetical conformation in which neither the bond angle ∠COC nor the rotational angles are restricted. This monotony of the curve suggests that the shoulder and the peak appeared in Curves 1 and 2 should not be attributed only to the regularity of the primary structure of the molecule.

Consequently we can conclude that heparin molecules have the conformation described by model 3, and that at least in part the helical conformation is maintained even in the solution. Model 3 was also supported by ATKINS and NIEDUZYNSKI (1977) on the basis of the X-ray fiber diffraction of heparin. The helix pitch, which is defined by the axial length per disaccharide, was estimated to be 0.75-0.85 nm from the position of the peak of the scattered intensity curve, which agrees with the value 0.84 nm obtained by ATKINS and NIEDUZYNSKI (1977).

Summary

X-ray small-angle scattering from heparin in solution was measured, and compared with theoretical curves based on various molecular models. Good agreement was obtained for the model consisting of α-D-glcosamine in the $^4C_1$ conformation and α-L-iduronate in the $^1C_4$ conformation. It was suggested that the helical conformation is maintained even in solution, at least in part. The helix pitch was estimated to be 0.75-0.85 nm.

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