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► To cite this version:

J. Guenet, C. Rochas, A. Brûlet. Molecular structures in biopolymers sols and gels. Journal de Physique IV Proceedings, 1993, 03 (C8), pp.C8-99-C8-102. 10.1051/jp4:1993819 . jpa-00252250

HAL Id: jpa-00252250

<https://hal.science/jpa-00252250>

Submitted on 1 Jan 1993

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Molecular structures in biopolymers sols and gels

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Abstract: The short range molecular structure of agarose in the sol state and κ -carrageenans in the gel state have been investigated by small-angle neutron scattering ($0.1 < q(\text{nm}^{-1}) < 2.5$). Agarose in the sol state possesses a rod-like conformation (minimum persistence length of 14 nm) with a mass per unit length consistent with a single helix. The existence of a double helix in the gel state is accordingly questioned. Agarose in sols from DMSO/WATER blends still exhibits the same rigidity yet the mass per unit length varies with solvent composition. It is surmised that this arises from strong complexation of agarose by DMSO which has consequences on the gelation propensity. Results obtained with κ -carrageenans gels are accounted for by an array of rigid rods consisting of two populations of differing cross-sectional radius.

INTRODUCTION

The present knowledge of the molecular structure of polysaccharides either in the sol state or in the gel state has been gained from polarimetry [1,2] together with X-ray diffraction [3,4]. Polarimetry experiments led to the conclusion that the chains had to behave as random flexible coils in the sol state. During the gelation process chains were thought to rigidify thanks to the formation of double helices that eventually aggregate to produce the final gel state. Strong evidence exists for iota-carrageenan to support the concept of double helices. Yet, in the case of agarose, the paucity of features in the X-ray diffraction pattern has led to a controversy [5]. Recently, Foord and Atkins [6] have suggested that the diffraction pattern can be as well interpreted with single helices. Clearly, additional data are necessary to cast some light on to the molecular structure of these polysaccharides. This is the aim of this note to report on data mainly gained from the sol state that may have a direct bearing on the structure in the gel state.

EXPERIMENTAL

Data were taken at LLB on PAXE small-angle camera in a q -domain ranging from $q = 0.1 \text{ nm}^{-1}$ to 2.5 nm^{-1} . To obtain the normalized intensity, $I_N(q)$, data were corrected for transmission, thickness and calibrated with an incoherent standard (*cis*-decalin). The blank sample contained the solvent plus an equivalent amount of protonated water so as to mimic the incoherent signal of the polysaccharide. The absolute intensity of the latter, $I_A(q)$, then reads:

$$I_A(q) = \frac{I_N(q)}{K} = C_H S(q) \quad (1)$$

in which C_H is the polysaccharide concentration, $S(q)$ its scattering function. K is the calibration constant which reads:

$$K = \frac{\left(a_P - \frac{v_P}{v_D} a_D\right)^2 \times 4\pi \times \delta_{dec} \times N_A \times T_{dec}}{g(\lambda) \times (1 - T_{dec}) \times m_P^2} \quad (2)$$

in which a_p and a_D are the scattering amplitudes and the molar volumes of the polysaccharide and of the solvent, respectively, δ_{dec} and T_{dec} the thickness and the transmission of the *cis*-decalin calibration sample, N_A the Avogadro's number, $g(\lambda)$ a correction factor, dependent upon the camera and upon the wavelength distribution, which has been presently determined by means of Cotton's method [7], and m_p the molecular weight of the polysaccharide residue.

In what follows rod-like chains or arrays of rod-like structures are dealt with for which the intensity in the present q -range approximates to [8]:

$$q^2 I_A(q) = C_p \left(\sum_{i=1}^{i=n} \pi \times q \times w_i \times \mu_{L_i} \times \frac{4J_1^2(qr_i)}{(qr_i)^2} + Const \right) \quad (3)$$

in which w_i , r_i , μ_i are the weight fraction, the cross-section radius and the mass per unit length, respectively, of the i^{th} rod-like structure.

RESULTS AND DISCUSSION

AGAROSE SOLS

Agarose conformation has been studied in mixtures of DMSO/water, from pure DMSO to pure water. In pure DMSO no gelation occurs whereas it does in pure water and in mixtures with a water content above 30%. Except for pure DMSO, all the sols have been studied at 70°C. A typical scattering pattern is represented in figure 1 which can be fitted by means of relation 3 with only one rod-like structure of radius 0.45 ± 0.15 nm. As the $1/q$ behaviour is seen in all the investigated domain we deduce a minimum persistence length of about 14 nm. Further, the scattering pattern virtually independent of the composition of the mixture suggests that agarose is an *intrinsically-rigid chain*. This conclusion is at variance with conclusions derived from polarimetry that suggested higher chain flexibility. However, polarimetry only indicates the presence of well-defined helices. The absence of well-defined helicity does not necessarily entails flexibility.

Figure 1: Scattering pattern (Kratky-plot) of agarose in deuterated DMSO at $T = 20^\circ\text{C}$. $M_w = 1.4 \times 10^5$, $C_p = 0.08$ g/cm³. The solid line is calculated from 3 with $n = 1$ and $r = 0.45$ nm. Here $K = 6.35 \times 10^{-4}$ as calculated with $d = 1.7$ g/cm³. Error bars stand for the uncertainties on the intensity of the blank sample.

As for the mass per unit length, it varies with solvent composition: the higher the water content, the larger the mass per unit length (see table 1).

In pure water we note that $\mu_L = 360 \pm 36$ g/nmxmole does not correspond to double helices ($\mu_L \approx 1000$ g/nmxmole) but to nearly extended chains of the 3_1 type as recently discussed by Foord and Atkins [6]. The only way to reconcile our data with the double helical structure would be to contemplate the formation of a strong agarose/water complex which would alter the value of the scattering amplitude. Recent experiments performed by means of the contrast variation method lead us to dismiss this possibility [8].

Taking into account the high rigidity of the agarose chain together with the mass per unit length determined here lead us to question the existence of double helices in the gel state. As a matter of fact, one may wonder how can very rigid chains intertwine to form a double helix whereas it would be easier for them to simply align. Our results give indirect support to those single helices contemplated by Foord and Atkins in the gel state.

In pure DMSO and in the other mixture we note that the mass per unit length is an apparent one as it is lower than the lowest possible value for an all-extended chain ($\mu_L \approx 330$ g/nmxmole). Here, unlike with agarose/water, the possible reason for an apparent value may be due to the formation of a strong DMSO-agarose complex for which the contrast factor would be lower than the one calculated for agarose in DMSO. This interpretation holds provided that the molar volume of the complex, v_C , differs from the sum of the molar volumes of agarose, v_A and DMSO, v_{DMSO} , respectively. A simple calculation shows that if $v_C = 0.95(v_A + v_{DMSO})$ with 2 to 4 DMSO molecules per agarose residue then one retrieves $\mu_L = 360$ g/nmxmole as in agarose/water sols.

Strong agarose/DMSO complexation may account for the absence of gelation in DMSO: the agarose-DMSO complex may simply possess a gelation temperature far lower than what can be achieved in this solvent. This viewpoint slightly differs from the former interpretation which, although considering the complexation of agarose by DMSO, states that gelation does not occur on account of the agarose chain flexibility.

Table 1: mass per unit length of agarose chains as a function of solvent composition

% DMSO/D ₂ O	100/0	30/70	50/50	30/70	0/100
μ_L (g/nmxmole)	230±23	256±20	293±20	341±20	360±36

κ -CARRAGEENANS GELS

A typical scattering pattern for κ -carrageenan gels at 20°C is represented in figure 2. A theoretical fit can be achieved by using relation 3 and considering two populations of rods with differing cross-sectional radii (see legend of figure 2). The meaning of this fit can be viewed as follows: the rods with the smaller cross-sectional radius correspond to carrageenan fibers while the rods with the larger radius correspond to the gels junctions where several of these fibres meet. Interestingly enough the weight average mass per unit length amounts to $\langle \mu_L \rangle_w = 1120 \pm 110$ g/nmxmole.

The fibres considered here correspond within experimental uncertainties to double helices. Indeed, the double helix as defined by Anderson et al.[3] possesses a radius of about 1.4 nm. Further, by assuming that i) $\mu_{Li} \sim r_i^2$ and ii) the junctions are made up of three double helices, the weight average mass per unit length calculated from the value of r_1 , w_1 and r_2 , w_2 with $\mu_{L1} = 933$ g/nmxmole is $\langle \mu_L \rangle_w = 1030$ g/nmxmole, a value in good agreement with the experimental one. The data derived here from small-angle neutron scattering are consistent with recent observations by cryoelectron microscopy of κ -carrageenan gels in vitrified ice [9].

CONCLUDING REMARKS

The results obtained with *agarose sols* show an unexpectedly rigid macromolecule whose conformation

Figure 2: Intensity scattered (in a Kratky-representation) by κ -carrageenans in the gel state. $C_p = 0.05 \text{ g/cm}^3$, $M_w = 1.13 \times 10^5$, salt = NaNO_3 $C_s = 5 \times 10^{-2} \text{ M}$. The solid line is calculated from 3 with: $r_1 = 1.5 \text{ nm}$ $w_1 = 0.94$ and $r_2 = 3.5 \text{ nm}$ $w_2 = 0.06$. Here $K = 3.39 \times 10^{-4}$ by considering the exchange of 3 protons and a density of 2.0 g/cm^3 for the polysaccharide.

is close to a near-extended chain. These results are altogether inconsistent with the notion of double helix in the gel state unless some mechanism is found for the chains to intertwine while changing their helicity instead of simply aligning. If such a mechanism is not found then one will have to consider seriously the occurrence of single helices in the gel state, good candidates being those described by Foord and Atkins[6].

Conversely, the results obtained with κ -carrageenan gels are consistent with the presence of double helices. Yet, the results presented here cannot distinguish between doubles helices or dimers of single helices, although the latter are said to be energetically not feasible[3].

Finally, it seems worth mentioning that similar results as those on κ -carrageenan gels have been obtained on *Poly[methyl methacrylate]* (PMMA) gels [10]. Stereoregular PMMAs possess a rare property among synthetic polymers: they form double helices. It may therefore indicate that the gelation behaviour is quite universal and, particularly, does not depend upon the solvent type as PMMA gels are produced in organic solvents.

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