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CONFORMATIONAL COMPARISON BETWEEN PURE AND CHEMICALLY MODIFIED AGAROSE

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ABSTRACT

In this work, we conduct a statistical study to compare the molecular conformations of pure agarose and agarose chem-ically modified. . It is to follow the process of their gelation through calculation of the rate of helix according to the tem-perature. Indeed, it is shown that the conformation of the helix is marked in the pur agarose gel by comparison with aga-rose chemically modified at the same temperature. It followed from there a study of the thermal hysteresis which confirms the reversibility of the two types of gel. Finally, we analyzed the addition of some organic compounds as co-solvents to understand their influence on the formation of the gel corresponding to the chemically modified agarose.

KEYWORDS: Helix-coil transition; Statistical mechanics; Chemically modified agarose sample; Thermal hysteresis; Cosol-vents; Thermoreversible gelation.

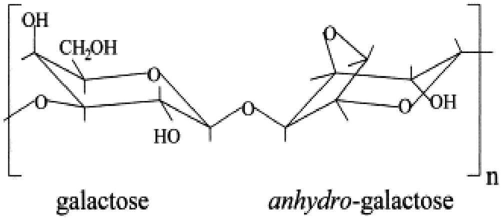
INTRODUCTION

Agarose is a natural polymer extracted from red marine alga. It is a polysaccharide frequently used in molecular biology for the separation of large molecules, in particular the DNA, by electrophoresis [1,2,8,12].

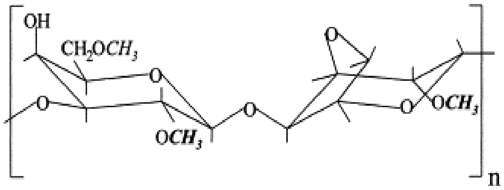
Agarose dissolves in cold water, and the aqueous solu tion undergoes a thermo-reversible sol–gel phase transition with thermal hysteresis [11]. The structure of the agarose is maintained by hydrogen bonds and may be disrupted by heating to a liquid state. The melting temperature is different from the temperature of gelation, in effect the gelation temperature of the agarose in the order of 40 ° C and its melting temperature is about 90 ° C [3,12]. The gelation of the biopolymer, that has been studied, among others, via calorimetry and a statistical study corresponds the transformation of the macromolecular chains in coil form (diluted) in a helical conformation (gel) [1,8].

In this work, we complement the statistical study which was devoted to the pure agarose initially to realize chemically modified agarose. We therefore, approach the process of gelling of this biopolymer in a series of binary solvent to describe the helix-coil transition to better understand the various parameters associated with this transition.

Agarose has been chemically modified by randomly replacing hydrogen of the hydroxyl groups OH by OCH_3 groups on the sites listed below [1,4,8].



Agarose modified chemically



This modification definitively removes the possibility of a hydrogen bond, while promoting the polarization of covalent bonds also leads to the appearance of fractional electric charges on different atoms. The groups OCH_3 may be crosslinked eventually leading to a network structure. The formation of a gel of agarose modified results from the combination of physical and chemical gelation [1,4].

We therefore analyzed the problem of gelation of this biopolymer in water and in a variety of binary solvent constituted by: water / dimethylsulfoxide (DMSO), water / dimethylformamide (DMF), Water / methylformamide (MF), and finally water / Formamide (FOR). In the four organic solvents pure, agarose does not gel. They are good solvents [1,8].

We used these four organic solvents to better understand their role and their effects on the process of sol-gel transition, which corresponds to the transition of conformation: helix-coil.

THE STATISTICAL MODEL STUDIED

In this work, we made a statistical analysis of the molecular conformation of chemically modified agarose. In this study, We consider a macromolecular chain established by N units (monomers) linked together, each unit can exist in two states: either the helical state (h) - or the coil state (p) [5,6,12].

To simplify, we can consider two statistical conformations:

- We have a mixture in a chain of states h and c in variable proportions: each chain is composed of n units in the helix form or coil form.
- Each macromolecular chain is either entirely in the form of coil units (c) or entirely in the helix units (h). We then represent symbolllically the only two possible forms [10,12].

The probability of finding the coil states (u) and helix state (w) are respectively defined by [6]:

$$u = \exp(-\frac{G(c)}{RT})$$

And

$$w = \exp(-\frac{G(h)}{RT})$$

Where G (h) and G (c) are the Gibbs free energy corresponding to configuration h and c.

The equilibrium constant of the reaction of transformation of a coil unit into a helical unit is given by:

$$s = \frac{u}{w} = \exp(-\frac{G(p) - G(h)}{RT}) = \exp(\frac{\Delta G_{c \to h}}{RT})$$

Introducing the change in enthalpy ΔH associated with the transition helix-coil transition which can be measured by calorimetry, we then assume that s is a function of the temperature that obeys the law of the van't Hoff type [9,12]:

$$\frac{d \ln s}{dt} = \frac{\Delta H}{RT^2}$$

By integration, we have:

$$\ln s = \frac{\Delta H}{RTT_t} \Delta T$$

Where $\Delta T = T - T_t$ and The transition temperature T_t is defined by: $s = 1$

In the case of the first hypothesis of a random mixture of coil and helix states, the total partition function for the chain is written as [6,12]:

$$Z(n,T) = \sum_{n_h, n_p} \exp(-G_{tot}(n_h, n_p) / RT) = \sum_{n_h, n_p} \prod uw$$

$G_{tot}(n_h, n_c)$ is the total Gibbs free energy of the chain in a configuration with n_c residues in the coil state and n_h in the helical state $n = n_h + n_c$.

Using the above expressions, we rewritten the partition function in the form [5]:

$$Z(n,T) = u^n (1 + \frac{u}{w}) = (u + w)^n$$

The ratio of helix χ is defined as the number of residues in helix conformation by the total number of residues in solution.

$$\chi = \frac{\prec n_h \succ}{n} = \frac{w}{n} \frac{\partial \log Z}{\partial w}$$

Finally:

$$\chi = \frac{s}{s+1}$$

For the second hypothesis, that the chain with two states, the form of Z (n, T) is extremely simplified, and it is written as [6,12]:

$$Z(n,T) = u^n + w^n = u^n (1 + s^n)$$

Therefore

$$\chi = \frac{1}{n} \frac{nw^n}{u^n + w^n} = \frac{s^n}{1 + s^n}$$

In this work, we opt for this last hypothesis.

RESULTS AND DISCUSSIONS

Comparison of fraction of helix between pure agarose and agarose chemically modified.

As a first step, we made a comparative statistical study of the molecular conformation of chemically modified agarose and pure agarose. This comparison consists of calculating the fraction of the two types of agarose. The fraction of helix contained in agarose for different temperature values with a worthless fraction of DMSO is shown in Figure 3 and via all the experimental results obtained in these studies are summarized in Tables I and II.

Table 1. The fraction of helix according to the temperature variation of the agarose modified chemically for two processes: gelation and melting

ΔT (K)	χ (fus)	χ (gel)
- 73,5	0,992	0,948
- 63,5	0,983	0,926
- 53,5	0,965	0,894
- 43,5	0,931	0,848
- 33,5	0,874	0,783
- 23,5	0,789	0,694
- 13,5	0,675	0,582
- 3,5	0,546	0,453
6,5	0,417	0,324
16,5	0,305	0,210
26,5	0,216	0,125
36,5	0,151	0,0683
46,5	0,105	0,0346
56,5	0,0738	0,0164
66,5	0,0515	0,0072

Table. 2 The fraction of helix according to the temperature variation of the pur agarose for two processes: gelation and melting

ΔT (K)	χ (fus)	χ (gel)
- 42,7	0,998	0,991
- 32,7	0,993	0,972
- 22,7	0,967	0,910
- 12,7	0,867	0,731
- 2,7	0,594	0,405
7,3	0,268	0,135
17,3	0,0897	0,0321
27,3	0,0276	0,0063
37,3	0,0086	0,0010

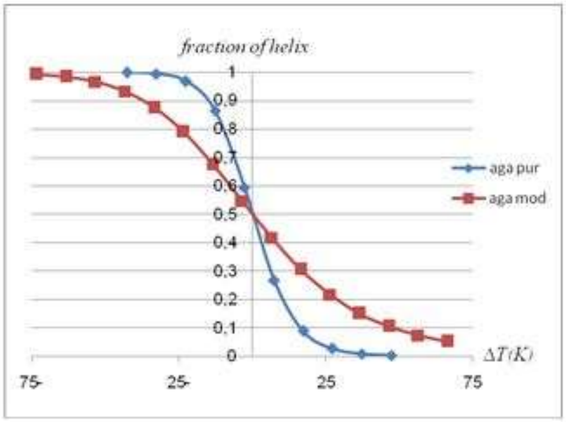


Figure 1. ***The variation of the fraction of helix according to a size of ΔT for a size of 400 residues and a concentration in DMSO: f_{DMSO}=0%.***

At low temperatures, the fraction of helix increases to a maximum value. On the other hand, for the high temperatures the fraction of helices decreases to a minimum value. The fraction of the variation of helix according to ΔT for a size of 400 residues and a concentration in DMSO.

The analysis for the two systems shows that the process of gelation of the modified agarose is significantly lower than that of the pure agarose. This decrease is explained by the fact that the OH groups are replaced by OCH_3 groups. This change eliminates the possibility of formation of hydrogen bonds. Thus OCH_3 destabilizes the helical structure and behaves like denaturing hydrogen bonds.

Study of thermal hysteresis

Thermal hysteresis is a phenomenon in which a physical quantity depends not only on the temperature but also the thermal history of the material. It is the area between the curve of gelation and the melting curve of the gel. Therefore the gel is not in a state of thermal equilibrium [6,10].

Figures 2.a and 2.b represents the variation of rate conformation in helix of the pure agarose and chemically modified according to the temperature variation for the two processes: gelation and fusion. We note that there is no reversibility when cooling: This is the phenomenon of thermal hysteresis.

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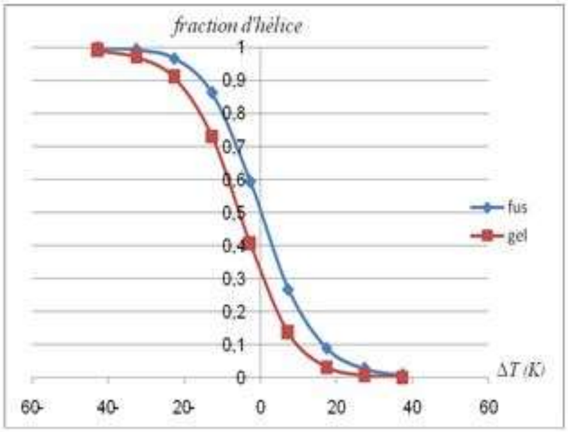

Figure 2.a. *The variation rate of the helix according to the temperature during gelling and fusion for pure agarose: Hysteresis.*

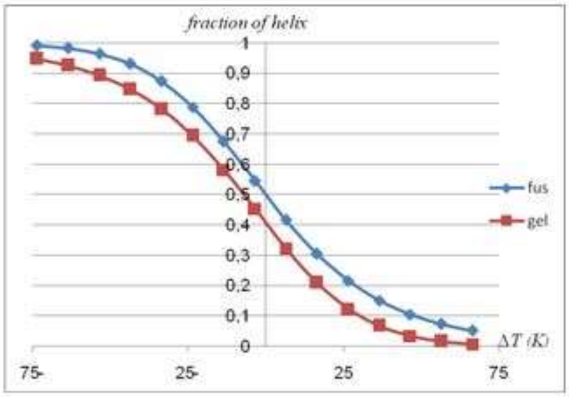

Figure 2.b. *The variation rate of the helix according to the temperature during gelling and fusion for chemically modified agarose: Hysteresis.*

For the agarose chemically modified (Fig 2.b) the gelling time is large compared to that which corresponds to the pure agarose. This difference amounts to the replacement of hydroxyl groups OH by groups OCH_3. Therefore, OCH_3 groups do not have the ability to form bonds with other atoms of solution for low temperature variations.

At the point extremum, the hysteresis disappears because of the influence of temperature and the ability to form hydrogen bonds within and between molecular.

The effect of some binary solvent on the fraction of helix

In this part, we discussed the process of gelation of this biopolymer in a series of binary solvent: Water / Dimethyl sulfoxide (DMSO), water / dimethylformamide (DMF), Water / methylformamide (MF), and finally Water / Formamide (FOR) through a statistical thermodynamic study to describe the helix-coil transition. Figure 4 shows the behavior of the fraction of the helix according to temperature variation for the three systems: modified agarose / DMF, modified agarose /MF and modified agarose / FOR, and all the experimental results obtained in these studies are summarized in Tables III.

Table 3. The variation of the fraction of helix according to ΔT for a series of solvent binary.

ΔT (K)	χ (DMF)	ΔT (K)	χ (MF)	ΔT (K)	χ (FOR)
-39,5	0,989	-41,3	0,995	-42,2	0,899
-29,5	0,962	-31,3	0,982	-32,2	0,833
-19,5	0,888	-21,3	0,933	-22,2	0,745
-9,5	0,727	-11,3	0,795	-12,4	0,640
0,5	0,487	-1,3	0,537	-2,4	0,526
10,5	0,264	8,7	0,272	7,6	0,417
20,5	0,125	18,7	0,113	17,6	0,322
30,5	0,057	28,7	0,044	27,6	0,243
40,5	0,025	38,7	0,017	37,6	0,182
		48,7	0,0072		

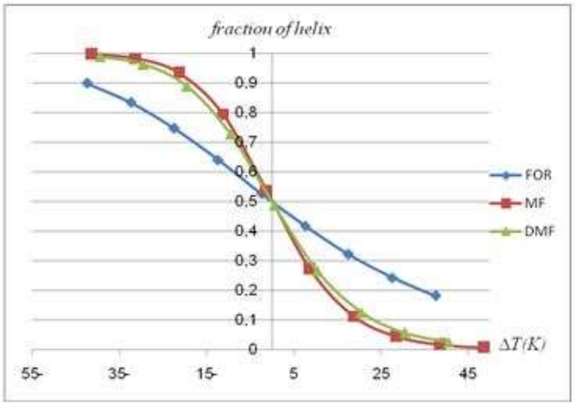

Figure 3. *The variation of the helix fraction according to the temperature for three chemical compounds FOR, MF and DMF. The molar fraction of each compound are respectively f_{FOR} = 0.2, f_{DMF}= 0.2 and f_{MF} = 0.2.*

This figure shows the helix-coil transition or order-disorder transition of the chemically modified agarose gel comprising n = 400 repeated units and fixing the molar fraction of the three compounds at f = 0.2.

Gradually, as the temperature decreases, the fraction of helices increases, thus leading to the decrease in entropy of the system because the interactions between the various atoms are reduced ie d. disorder decreased. We look through this figure as gelation is less marked for the modified agarose / FOR compared to other systems. This difference is explained by the fact that the atoms of formamide (FOR) does not have the possibility to establish hydrogen bonds with their neighbors, in other words, the formamide are less cooperative with the atoms of the solution.

CONCLUSION

In this work, we studied the thermoreversible gelation of chemically modified agarose in binary aqueous solvents (dimethylformamide and a series) by a statistical thermodynamic analysis. The results presented in this paper clearly show the role of the binary solvents in the thermoreversible gelation of chemically modified agarose. This organic solvent allows the gelation of a part of the material that could not gel in pure water. As has been discussed, this effect is not due to the special interactions that may exist between water and the organic solvent. As a matter of fact, due to bond polarization, CH_3 groups tend to bear a positive electric charge and can thus interact with the negatively charged oxygen. The organic solvent therefore helps to replace the missing hydrogen bondings between the former OH groups.

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