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A statistical study of the helix-coil transition of agarose

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Abstract:- In this work, we present a systematical of statisticals study of the helix-coil transition of agarose. The statistical study of the agarose gel consists in calculating "the equilibrium constant" which is the ratio of the probability of finding the two conformations and also determining the various rate of the state through the coil-helix transition that is found in the agarose gel for a transition temperature T_t=78.8°C and concentration C_p=15%. According to the experimental study which was made on pure agarose, the variation of the content of helix and the equilibrium constant is given from the law of van't Hoff. For agarose in the independent-residue model, a change in s from 0.978 to 1.021 leads to a change in fh from 0.494 to 0.505. For a 300 residue agarose in the two-state model, the same change in s leads to a change in fh from 0.999 to 0.0291. Finally, the effect of dimethylsulfoxide (DMSO) on the helical fraction depending on the temperature is studied, and we discuss the effects of the solvent composition on the helix-coil transition of an agarose chain.

Keywords:- Helix–coil transition; statistical mechanics; agarose gel;Solvent effect; Dimethylsulfoxide (DMSO)

I. INTRODUCTION

Agarose is a natural polymer extracted from the red marine alga. It is one of the two major components of the both polygalactoside agar, and agaropectine. Agarose is frequently used in molecular biology for the separation of large molecules, especially DNA, by electrophoresis [1,3].It forms thermoreversible gels when dissolved in water, and is normally insoluble in organic solvents, exept a few exceptions such as DMF and DMSO. In those solvents, it cannot form gels unless a certain amount of water is added. Apparently, the structure of water plays a decisive role to induce gelation [3]. Agarose is a linear polysaccharide composed of repeating basic unit: the agarobiose.

The basic disaccharide repeating units of agarose consists on (1,3) linked β-d-galactose (G) and (1,4) linked α-l-3,6-anhydrogalactose (A) (Fig. 1) (Araki, 1966) [2].

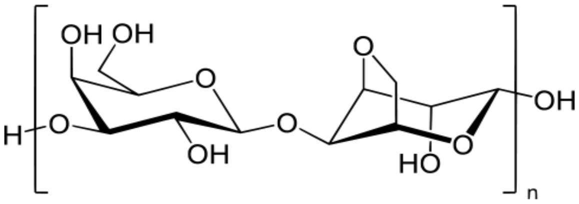

Fig.1: The structure of an agarose polymer [1].

A characteristic feature of agarose is that the gels show a large thermal hysteresis, attributed to the formation of large aggregates that remain stable at temperatures much higher than those at which the individual helices reform on cooling [3].

Polymer molecules in solution can be found in many different geometric conformations, and there exist a variety of experimental methods (optical rotation…), for obtaining information about these conformations. The two important kinds of conformation of an agarose chain are the helix and the random coil.

When the temperature is raised or the pH is changed, the helix becomes disordered into random coil states. This phenomenon is known as helix-coil transition (Fig. 2). This transition has been extensively studied, both experimentally and theoretically, as a model for conformational transition in biopolymers and as a way to obtain information about the intermolecular forces which stabilize biopolymers structure [4].

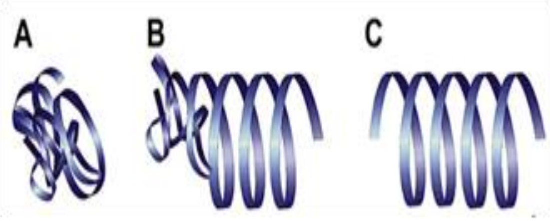

Fig 2: The helix-coil transition over time [16].

The most interesting feature of the helix–coil transition in biopolymers is its cooperation which is a manifestation of long-range interactions along the chain. Many factors can influence the cooperation of transition. It depends, for example, on the chain length, ionic strength of the solution and the pH, etc [5].

The usual way of describing the helix–coil transition is to find the dependence of the degree of helicity χ, on the external parameter (e.g. the temperature); χ, is defined as the average fraction of the bounded pairs: $\chi = \prec n_h \succ / n$; where n is the total number of base pairs and $\prec n_h \succ$ is the average number of the bounded pairs. The graph of this dependence is called a ‘‘transition curve’’. The temperature at which χ=0,5 is called transition point (T_t) and is one of the characteristics of the transition process [5].

Many theoretical models for this transition have been developed, beginning with the pioneering work of Schellman (1958), Zimm and Bragg (1959), and Lifson and Roig (1961) more than 50 years ago. In particular, Zimm and bragg developed a theory of the helix-coil transition for polypeptides in terms of the solution of a strongly-biased Ising chain [6].

A statistical procedure used in this work is to determine the two parameters of the transition: The first parameter is the equilibrium constant s of the reaction of transformation of a coil unit into a helix unit. The temperature dependence of s is given by the Van’t Hoff equation. The second parameter is the fraction of helix that is found in the agarose gel. In this paper, we discuss the effects of the solvent composition on the helix-coil transition of an agarose chain [7,8].

II. EXPERIMENTAL

A. Materials

All experiments of thermal analysis were performed on a METTLER TA 4000 calorimeter. This comprises a measuring cell METTLER DSC30, associated with a control module METTLER TC11 managed by a METTLER GRAPHWARE TA 72 operating program. The liquid nitrogen supply system achieves more than 20 degrees K.

The DSC30 measuring cell includes the reference sample to be analyzed and which is contained in a hermetically sealed aluminum crucible. The crucible of reference must have a similar weight and shape of the sample crucible. Usually, the weight of the crucible reference equilibrated by adding a quantity of solvent equal to the amount of gel in the sample crucible. This avoids the problems of thermal inertia between the two crucibles.

Before any quantitative (or qualitative) measurement, the calorimeter must be calibrated. The system TA4000 is provided with a list of parameters required to set the temperature of the furnace to the extent thereof and conversion of the measurement signal in the heat flow dH/dt. These parameters must be experimentally determined and recorded manually in the configuration list [1,9].

B. Preparation of sample.

All agarose samples we used are in a white powder's form.

After putting the solvent into the vial, we added the polymer which is weighed beforehand to obtain the desired concentration. The later is added slowly by agitating the whole with a magnetic stirrer.

At the end of this operation, the vial is placed in a bain-marie without stopping the agitation. This phase is extended more or less long depending on the concentration of the sample. When the entire polymer is dissolved, that is to say, when the solution becomes homogeneous, it is allowed to cool to a temperature preparation for gel formation. The gels formed are left for 24 hours at room temperature before handling. This preparation procedure allows to obtain gel concentration very accurate but limited to a maximum of 7%. Indeed, beyond this value, it becomes almost impossible to disperse and dissolve the agarose in the solvent [1,9].

III. STATISTICAL MODEL

In statistical way to describe the behavior of a chain and its transition of conformation, a long chain molecule consisting of n linked units is considered, each unit being able to exist only in to states, the helical state (h) and the random coil state (c). The units, called residues consist of several atoms and if the dihedral angles (angles of internal rotation about its single bonds) are those characteristic of the helix, it will be in the state (h); otherwise it will be in the state (c) [10, 11].

In this study, we can consider two statistical assumptions:

- 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.

  …… cchchcchhh……

- Each macromolecular chain is either entirely in the form of coil units (c) or entirely in the helix units (h). We then represent symbolically the only two possible forms [10].

  …..hhhhhh…. or …..cccccc….

The probability of being in this state will be defined for a chain unit in the coil state c by [11]:

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

(1)

And for a unit in the helical state h by:

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

(2)

Where G (h) and G (c) are the Gibbs free energy corresponding to configuration h and c.
The ratio is defined by [11]:

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

(3)

It defines the equilibrium constant of the reaction of transformation of a coil unit into a helical unit: c↔h.
Where ΔH and ΔS are corresponding enthalpy and entropy contribution, and R the universal gas constant.

Here the statistical weights have been normalized with respect to the all-coil states. The physical behavior of this assignment is as follows: The hydrogen bond determines the stability of the helix and hence:

$$w \sim \exp(-\frac{E_H}{RT})$$

(4)

With E_H being free energy of formation of the hydrogen bond.

The internal rotations of the chains make up the free energy of the random coil, and thus disordered, is essentially entropic in nature:

$$u \sim \exp(\frac{S}{R})$$

(5)

Here s is a parameter whose value determines the most probable state for a chain element at temperature T:

- $s < 1$ The coil form is favored.
- $s = 1$ Both configurations are equally probable.
- $s > 1$ The helical form is favored.

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 a law of the van't Hoff type [11,12]:

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

(6)

The transition temperature T_t is defined by: $s = 1$. By integration, we have:

$$\int_{T_t}^{T} d\ln s = \left[-\frac{\Delta H}{RT}\right]_{T_t}^{T}$$

(7)

Setting $\Delta T = T - T_t$, we thus have:

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

(8)

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 [11]:

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

(9)

$G_{tot}(n_h,n_c)$ 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 (1) and (2), (9) is rewritten as [10]:

$$Z(n,T)=\sum_{n_h,n_p}^{\infty}\frac{n!}{n_h\,!(n-n_c)}w^{n_h}u^{n-n_h}$$

(10)

Or

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

(11)

The helical fraction χ is defined as the number of residues in helical conformation by the total number of residues in solution [11].

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

(12)

Therefore,

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

(13)

With $s=\exp(\frac{\Delta H}{RTT_t}\Delta T)$

If we apply the second hypothesis of the two-state chain, the form of Z (n,T) is extremely simplified; it is written as [11]:

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

(14)

The equilibrium constant and the helix content that is

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

(15)

IV. RESULTS AND DISCUSSION

a. Helix content

Figure 3 shows the behavior of the average helical fraction as a function of temperature of the solution and all the experimental results obtained in this work are summarized in Table I.

Table I: The equilibrium constant and the fraction of helix as a function of temperature

For n=30						
ΔH_t(j/g)	T_t(K)	T(K)	ΔT(K)	s	χ	log s
		333,15	−18,8	1,899	0,655	0,278
		338,15	−13,8	1,590	0,613	0,201
		343,15	−8.8	1,338	0,572	0,126
		348,15	−3.8	1,132	0,530	0,053
		353,15	1,2	0,962	0,490	−0,016
		358,15	6,2	0,821	0,450	−0,085
		363,15	11,2	0,704	0,413	−0,152
		368,15	16,2	0,606	0,377	−0,217
		373,15	21,2	0,524	0,343	−0,280

−3,6	351,95	For n=300				
		333,15	−18,8	610,042	0,998	2,785
		338,15	−13,8	103,357	0,990	2,014
		343,15	−8.8	18,441	0,948	1,265
		348,15	−3.8	3,457	0,775	0,538
		353,15	1,2	0,679	0,404	−0,167
		358,15	6,2	0,139	0,122	−0,854
		363,15	11,2	0,030	0,029	−1,522
		368,15	16,2	0,006	0,006	−2,171
		373,15	21,2	0,001	0,001	−2,804

At low temperatures ie. $\Delta T < 0$, the agarose chains adopts a helical conformation, these coils are associated by hydrogen bonds to form pseudocristalline areas leading to the formation of a thermoreversible gel.

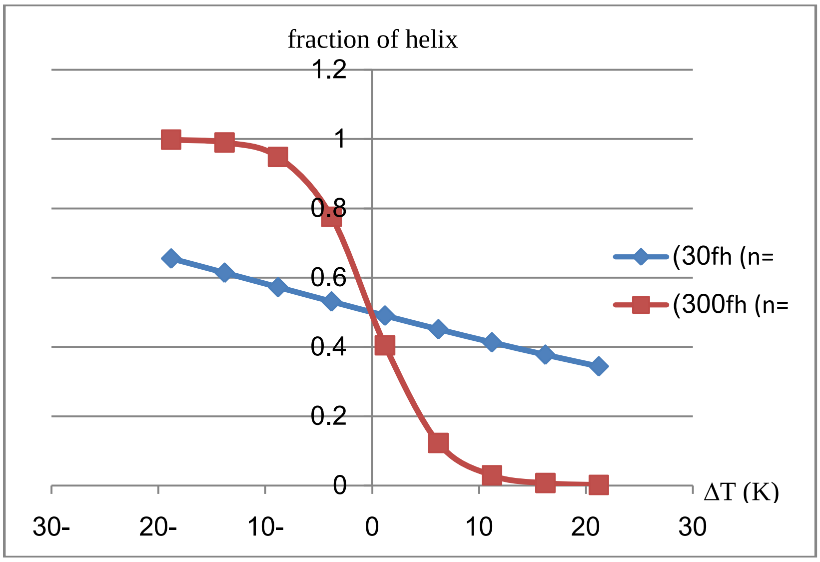

Fig. 3: The variation of the helical fraction according to the temperature of the lengths n = 30, n=300. The theoretical curve is obtained from relation by choosing T_t = 78.8°C, an agarose concentration C_p=15% and the molar fraction of DMSO f_{DMSO} = 0%.

The charges on the polymer are screened and repulsion is decreased, which contributes to the lowering of the free energy of the helix, around critical temperature $T_c = 318{,}15K$, the average helical fraction is maximum and increases with the size of the chain as shown in Figure 1. For small chain lengths, the H-bonds cannot form long overlapping cooperative strings, and as a result the helix content is far from unity. This transition is all the more marked as the number of unit's n of the string is greater because the portion of helix is proportional to the number of unit repetition of a chain [11].

At high temperature ie. $\Delta T > 0$, the coil form is favored, or each agarose chain is folded round and does not hydrogen bond to the propellers, and the effects on the size of the chain play a role in the behavior of the helical fraction, and that the free energy of binding becomes more positive than the helicity of the chain decreases while the entropy favors its formation. So the agarose gel to melt only and finally we found the coil state.

The entropy of transition

We see that a transition occurs at s=1, or when half of the molecules are in the h and c states. This transition corresponds to a temperature called the transition temperature T_t. This is the temperature where $\Delta G_{c\to h} = 0$. This gives [8, 13]

$$\Delta S_{c\to h} = \frac{\Delta H_{c\to h}}{T_t}$$

However from experimental values: $\Delta H_{c\to h} = -3,6\, j/g$ and $T_t = 351,95K$, so

$$\Delta S_{c\to h} = \frac{-3,6}{351,95} = -10,22\times 10^{-3}\, j.g^{-1}.K^{-1}$$

b. The equilibrium constant

One of the most useful parameters to write the helix-coil transition is the equilibrium constant s, which is related to the enthalpy of transition. The behavior of this parameter as a function of the helical fraction is shown in **Figure 4.**

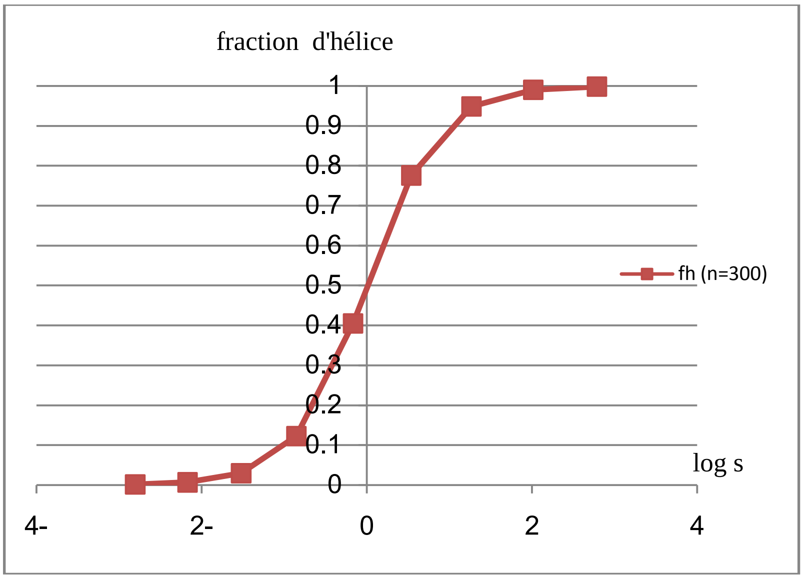

Fig. 4: The variation of the helical fraction versus log s to the length of n=300. The theoretical curve is obtained from relation by choosing T_t = 78.8°C and an agarose concentration C_p=15%.

For a given temperature, the longer agarose chains have a higher value of s. The only the exception is for the chains with small value of n, the transition near $s=1$ is milder, which is a general feature of the phase transition, and it has been deter- mined from the helical fraction, and the latter is low at low temperature due to fraying of the helix ends.

For $s>1$, the helical form is favored; leading to the formation of a rigid agarose gel, then the entropy does not favor its formation. Around $s=1$, a more abrupt phase transition which is in agreement with the experimental observation is founded [12,13]. At the lowest temperatures sampled, s is between 1.26 and 2.8 for n=300. As the temperature increases the value of s decreases, passing through 1 to 0 to reach the coil conformation only because the chain contains flexible areas.

c. The effect of dimethylsulfoxide (DMSO) on the helix content.

Dimethylsulfoxide (DMSO) is an organosulfur compound with the formula $(CH_3)_2OS$. This colorless liquid is an important polar aprotic solvent that dissolves both polar and no polar compounds and is miscible in a wide range of organic solvents as well as water [14].

This compound is used in this work to determine their effect on the structural conformation of agarose gel, i.e. the transition between the helix state and the coil state. Figure 3 shows the helix content in the agarose gel for different mole fraction DMSO depending on the temperature variation for a chain of length n=300 residues [15] and all the experimental results obtained in this work are summarized in Tables II and III.

Table II: Enthalpy of transition for different fraction of DMSO.

f_{DMSO}	$\Delta H_t(j/g)$	$T_t(K)$
0	$-3,6$	$351,95$
$0,1$	$-5,2$	$355,85$
$0,2$	$-5,7$	$353,95$
$0,37$	$-4,1$	$338,65$
$0,5$	$-2,4$	$318,85$

Table III: Helix content for different fraction of DMSO.

$\mathrm{T(K)}$	$\chi(\mathrm{f_{DMSO}} = 0)$	$\chi(\mathrm{f_{DMSO}} = 0,1)$	$\chi(\mathrm{f_{DMSO}} = 0,2)$	$\chi(\mathrm{f_{DMSO}} = 0,37)$	$\chi(\mathrm{f_{DMSO}} = 0,5)$
333,15	0,998	0,999	0,999	0,902	0,026
338,15	0,990	0,999	0,999	0,549	0,008
343,15	0,948	0,997	0,996	0,146	0,002
348,15	0,775	0,973	0,951	0,024	0,0008
353,15	0,404	0,775	0,599	0,003	0,0002
358,15	0,122	0,260	0,109	0,0006	0,0001
363,15	0,029	0,036	0,010	0,0001	$3{,}708E-05$
368,15	0,006	0,004	0,001	$2{,}082E-05$	$1{,}367E-05$
373,15	0,001	0,0005	0,0001	$3{,}967E-06$	$5{,}182E-06$

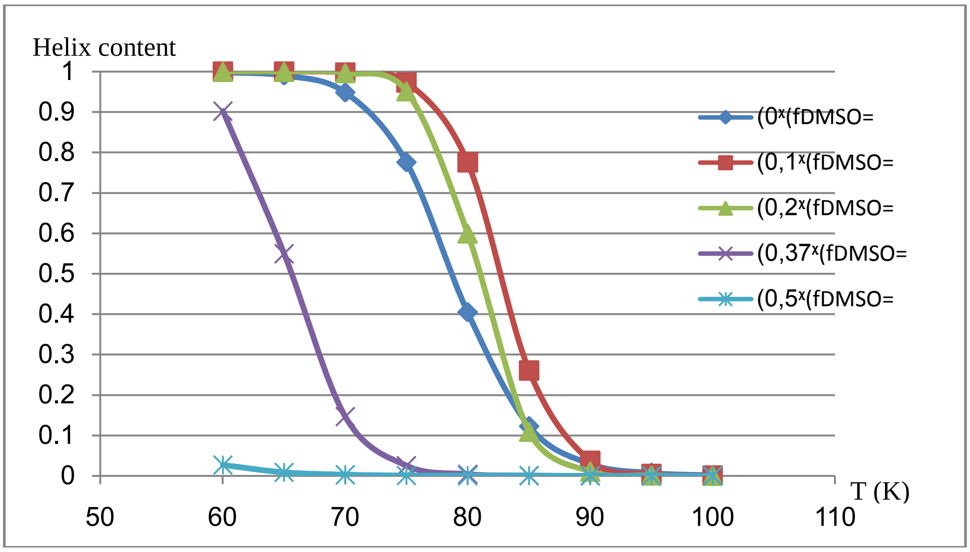

Fig. 4: The effect of DMSO on the helix content depending on the temperature

It Figure show typical transition curves for Agarose in DMSO mixtures.

The agarose in these solvent mixtures undergoes a transition from coil to helix with raising temperature [17] and the structural transition induced in DMSO depends on the concentration of the solvent. A low concentration of DMSO (< 0.1), the helical structure of agarose is preserved at low temperature. For large concentrations of DMSO, the fraction of helix decreases when the temperature variation increases as the concentration increases 0,1 to 0,5. In particular, to a critical concentration (near 0,1), the fraction of helix presents a maximum according to the temperature. This fraction also shows a sharp transition and a lower than unity. For example with T=75°C, the helix content equal to 76%; 97.31%; 95,17%; 2,48%; 0% have corresponds respectively to the concentration in DMSO 0; 0,1; 0,2; 0,37; 0,5. Therefore the data of the figure shows that the fraction of the helix decreases with the increase the concentration of DMS O. These results are consistent with the conclusion that DMSO desastabilise helical state i.e. the intramolecular hydrogen bonds decreases, above which the helical conformation is gradually lost. These results also show that the helix-coil transition associated with the gelation has a thermal hysteresis: the agarose gel returns to the ground state by heating.

V. CONCLUSIONS

The phenomenon of helix-coil transition in biopolymers has been the object of intensive research for a very long time. This transition can be observed by various physical means, optics by example, it occurs in a very narrow field of the external medium conditions (temperature, pH, concentration…).

We have presented an approach that combines models of statistical mechanics of the agarose gel. We have applied this approach to the helix-coil transition for the analysis of the conformational structure of the biopolymer. The latter is known to undergo a helix-coil transition in solvent mixtures merely an active solvent such as DMSO. Indeed, agarose has a number of OH groups that may interact in the same way as water and DMSO. The expressions are calculated for the equilibrium constant, s, of the helix forming propeller and fraction, they are used to analyze the given agarose obtained depending on the temperature and solvent composition.

We used a model of a single chain to study the balance of helix-coil transition i.e. the determination of the equilibrium constant s between the coil state and helix state.

In the last part of this work, we used a polar organic solvent (DMSO) at different mole fraction and we discussed the effects of the composition of the solvent on the coil-helix transition. We used a simple model of an agarose chain which includes 300 residues to demonstrate that improving the capacity of hydrogen bonding of the solvent can make the transition cooperative months.

Finally, in the next work we will tackle the problem of gelling agarose by studying the rheological properties of the gel.

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