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SHORT COMMUNICATION

# Study of the sodium dodecyl sulphate–protein complexes: evidence of their wormlike conformation by treating them as random coil polymers

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**Abstract** The Kuhn statistical segment length, A, was determined for the sodium dodecyl sulphate (SDS)–protein complexes in two binding levels of SDS adsorbed onto the protein. These complexes are treated as random coil polymers. For the SDS–protein complex of binding level 1.4 g SDS per gram of protein, the value of A was found equal to 76 and 37 Å in 0.01 and 0.1 M ionic strength, correspondingly. For the complex of binding level 0.4 g SDS per gram of protein, the value of A was found equal to 30 Å. The calculation was based mainly on two equations (Eqs. 4 and 5; Dondos and Benoît, Polymer 18:1161, 1977; Dondos and Staikos, Colloid Polym Sci 273:623, 1995, correspondingly). Our findings are supported by gel permeation chromatography results, drawn according to the "modified universal calibration" and suggest that these complexes should be considered as wormlike polymers presenting a considerable draining effect.

**Keywords** Sodium dodecyl sulphate–protein complexes · Statistical segment length · Wormlike polymers · Draining effect · Flory's parameter Φ · Gel permeation chromatography

## Introduction

Many works have been devoted on the morphology study of the sodium dodecyl sulphate (SDS)–protein complexes concerning the so-called gross morphology. Nevertheless, there is not a general accordance on the morphology of these complexes [1-4]. On the opposite, there is an agreement that there are two kinds of such complexes related with two binding levels of SDS with the polypeptide, 0.4/1 and 1.4/1, i.e., 0.4 and 1.4 g of SDS per gram of protein.

In the present work, we study the chain flexibility of the above complexes by determining their statistical segment length and by taking into account the quantity of the SDS adsorbed onto the protein to determine their molecular weight. We use an equation previously proposed for the determination of the unperturbed dimensions of flexible and semi-flexible macromolecules [5] in conjunction with an equation proposed for the determination of the Flory′s parameter Φ in systems exhibiting an important draining effect, due to their rigidity [6]. Moreover, by taking into account this Φ determination, we have a satisfactory explanation of the gel permeation chromatography (GPC) results.

## Procedure

The statistical segment of a macromolecular coil expressing, in general, the flexibility of the macromolecular chain, is determined by various physicochemical methods when the polymer is dissolved in a Θ solvent. In such a case, the intrinsic viscosity $[\eta]$ of the polymer varies with the molecular weight, $M$, of the polymer according to the equation

$$[\eta] = K_\Theta M^{1/2} \tag{1}$$

where $K_\Theta$ is the unperturbed dimensions parameter.

In cases where a Θ solvent is not available, some graphical methods have been proposed in order to obtain $K_\Theta$ from measurements conducted in non-Θ solvents. Between these

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methods, the most popular is the Stockmayer–Fixman–Burchard (SFB) equation [7, 8]:

$$\frac{[\eta]}{M^{1/2}} = K_\theta + 0,51\Phi BM^{1/2} \quad (2)$$

In this equation, Φ is the Flory′s parameter and $B$ a parameter dependant on the polymer–solvent interactions. By plotting $[\eta]/M^{1/2}$ versus $M^{1/2}$ and by extrapolation of the straight line obtained at $M$=0, the value of $K_\Theta$ is obtained. From the value of $K_\Theta$ and using the well-known equation

$$A = \left(\frac{K_\theta}{\Phi}\right)^{2/3} M_L \quad (3)$$

we obtain the value of the Kuhn statistical segment length, $A$. $M_L$ is the molecular mass per unit length.

The following equation was also proposed in order to obtain the $K_\Theta$ parameter from values of $[\eta]$ obtained in good solvents (Dondos–Benoît equation) [5]

$$\frac{1}{[\eta]} = -A + \frac{1}{K_\theta M^{1/2}} \quad (4)$$

Plotting $\frac{1}{[\eta]}$ versus $\frac{1}{M^{1/2}}$, we obtain a straight line with a slope giving the inverse of the unperturbed dimensions parameter $K_\Theta$. The value of −A expresses the polymer–solvent interactions. As we will see later, Eq. 4 gives the $K_\Theta$ value in cases where the SFB equation fails.

According to Eq. 3, the statistical segment length depends not only on $K_\Theta$ but also on the value of the Flory′s parameter, Φ. This parameter has been, in general, considered to be constant and equal to $2.6\times10^{23}$ cgs. This is generally valid for flexible polymers in organic solvents where the model of the semi-permeable to the solvent coil is applicable, and the draining effect is negligible. In the case of semi-flexible polymers, where a draining effect appears, Φ value is not anymore constant, but it decreases with increasing chain rigidity, as it has been shown in the Yamakawa–Fujii work [9]. The appearance of the draining effect, for these polymers, is also expressed by the increase of the value of the exponent of the Mark–Houwink–Sakurada (MHS) equation, a, and is also related with a decrease of Φ. On the basis of the results obtained by the treatment proposed in the above work, we have proposed the equation [6]

$$\Phi = 0.52 \times 10^{23} a^{-2.32} \quad (5)$$

This equation relates the value of Φ with the draining effect, appeared in the case of the semi-flexible polymers, by means of the value of the exponent a which deviates from the value 0.5, to higher values, as the draining of the chain increases. Equation 6 has also obtained a theoretical support [10, 11].

Moreover, the GPC will be used for the study of the SDS–protein complexes. More precisely, we will use the "modified universal calibration" [12] instead of the "classical" one [13]. In the classical universal calibration, the hydrodynamic volume of the coil is expressed by the product $[\eta]M$ according to the Fox–Flory equation, considering the Φ value as a constant

$$[\eta] = \frac{\Phi < R^2 >^{3/2}}{M} \quad (6)$$

In the "modified universal calibration" [12], the hydrodynamic volume is expressed by $[\eta]M/\Phi$, according to Eq. 6, and the value of Φ is obtained from Eq. 5.

Plotting $\log([\eta]M)$ as a function of the elution volume, $V_e$, by following the "classical" universal calibration, we obtain different curves from different polymers when these polymers are semi-flexible presenting a draining effect [14]. On the contrary, the curves obtained with such polymers plotting $\log([\eta]M/\Phi)$ versus the elution volume, where Φ is obtained from Eq. 5, coincide in one [12].

All the above equations and treatments are proposed for the polymers presenting random coil behaviour in solution.

## Results and discussion

The viscometric results on the SDS–protein complexes have been obtained from the article of Reynolds and Tanford [1]. They have measured the intrinsic viscosity at binding level of 1.4 g of SDS per gram of protein and at binding level of 0.4 g of SDS per gram of protein. The molecular weight of the polypeptide chains (chains of denaturated proteins) was obtained in guanidine hydrochloride (GuHCl).

In the following, we will not use the molecular mass determined in GuHCl but a molecular mass calculated by taking also into account the adsorbed SDS quantity. In the binding level of 1.4 g SDS per gram of protein, by taking into account that the mean molecular mass of each peptide group is equal to 110 [15], it occurs a molecular mass equal to 264 for the monomer unit of the SDS–protein complex. In the case of the binding level 0.4 g SDS per gram of protein, the molecular mass of the monomer unit of the complex is equal to 154.

In Fig. 1, we present the viscometric results of [1] for the 1.4/1 binding level (curve A) by using the complex mass, instead of the peptide mass, according to Eq. 4. Following this presentation, we obtain a $K_\theta$ value equal to $5.4\times10^{-2}$ ($cm^3$ $g^{-3/2}$ $mol^{1/2}$). Curve B in Fig. 1 represents the viscometric results obtained for the 0.4/1 binding level. The $K_\Theta$ value obtained for this SDS–protein complex equals to $1.85\times10^{-2}$. Here, we have to point out that the

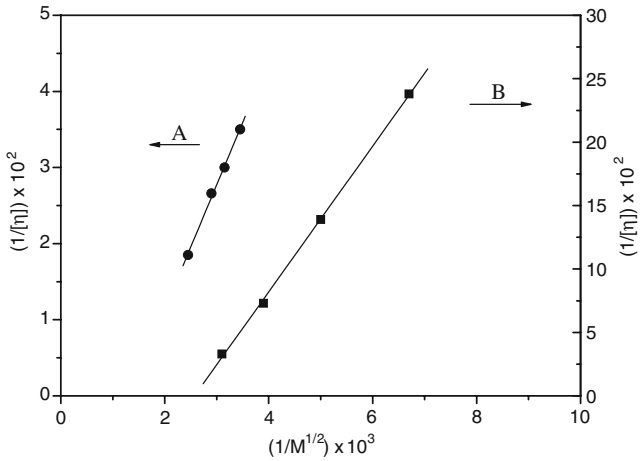


**Fig. 1** Variation of $1/[\eta]$ versus $1/M^{1/2}$ for the SDS–protein complexes: curve *A*, 1.4/1 binding level, curve *B*, 0.4/1 binding level (results from [1])

complexes of the same binding level but of different molecular mass are considered as homologous series as the protein polypeptides [15] or the fractions of a polymer.

In order to find the Φ value from Eq. 5, we need the MHS exponent a values. For this purpose, we have taken into account the four higher molecular weight proteins studied in [1] for the 1.4/1 complex, and we have found a=1.04 and from Eq. 5 $\Phi=0.48\times10^{23}$ cgs. In order to find the statistical segment length, from Eq. 3, we moreover need the value of the mass per unit projection length, $M_L$. For the complex 1.4/1, $M_L=69.5\times10^8$ Da cm$^{-1}$ or 69.5 Da Å$^{-1}$ ($M_L$=264 Da/$3.8\times10^{-8}$ cm, where $3.8\times10^{-8}$ cm is the projection length of the polypeptide repeating unit).

On the basis of the values of the above parameters, we obtain from Eq. 3 $A=76\times10^{-8}$ cm or $A$=76 Å. This result shows that the 1.4/1 complex can be considered as a wormlike polymer in accordance also with the high value of a.

From the MHS representation given in [1] for the 0.4/1 complex, we have a=1.25 and from Eq. 5, $\Phi=0.31\times10^{23}$ cgs. The $M_L$ value for this complex is calculated to be equal to $40.5\times10^8$ cm$^{-1}$ (154 Da/$3.8\times10^{-8}$ cm). By applying Eq. 3, we obtain $A=30\times10^{-8}$ cm or $A$=30 Å. This result shows that the chain of this complex is more flexible than that of the 1.4/1 complex. This behaviour is explained by the fact that the SDS adsorbed is less than in the 1.4/1 complex, i.e. about a quarter instead of a half SDS molecule per peptide repeating unit so that the charge of the complex chain is the half of the 1.4/1 charge. Moreover, the ionic strength of the 0.4/1 system is higher than that of the 1.4/1 system, 0.5 M instead of 0.024 M, correspondingly. This ionic strength increase, in the case of the 0.4/1 system, should provoke a shrinkage of the complex coil due to the charge shielding effect [16].

Using the viscometric results of Rao and Takagi [17] and applying Eqs. 4 and 5, we have found a value for the statistical segment length, $A$, of the complex SDS–protein of 1.4/1 binding level equal to 37 Å. This value is much lower than the value of 76 Å obtained with the results of [1] treated in Fig. 1. We attribute this difference to the higher ionic strength used in [17], 0.1 M, as it compares with the considerably lower ionic strength used in [1], 0.026 M.

Plotting $[\eta]/M^{1/2}$ versus $M^{1/2}$ for both complexes of [1], according to Eq. 2, we do not obtain acceptable values for $K_\theta$, as for the 1.4/1, $K_\theta=0$ and for the 0.4/1, $K_\theta<0$. It has been shown in previous works that the SFB equation does not give acceptable $K_\theta$ values for the wormlike polymers, but only if they have a very high molecular weight [18, 19].

In the following, in Fig. 2, we present the so-called modified universal calibration of GPC [12] for a number of native proteins and for the 1.4/1 SDS–protein complexes. This study is based on the GPC results obtained by Mori and Barth [20] who, by presenting their results as log$M$ versus retention volume and by using the same columns, have obtained two different curves for the native proteins and the SDS–protein complexes. More precisely, the curve corresponding to the SDS–protein complexes is obtained when the complexes are founding in a 0.01 M ionic strength and on a SDS concentration equal to 3.5 mM [20].

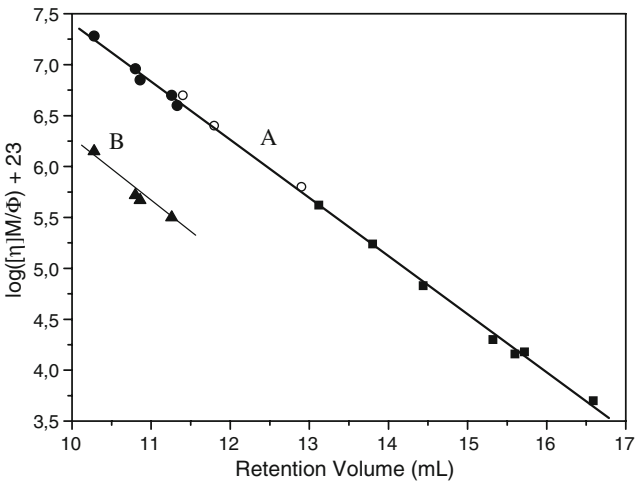


**Fig. 2** Variation of $\log([\eta]M/\Phi)$ versus retention volume according to the modified universal calibration [12]. Curve *A*: native proteins (*filled squares*; the Φ values for each native protein are from [22]); SDS–protein complexes of 1.4/1 binding level, from [1] (*filled circles*; the ionic strength is 0.02 M, the molecular weight of the complexes is corrected, and the Φ value is equal to $0.48\times10^{23}$ obtained from Eq. 5); SDS–protein complexes of 1.4/1 binding level, from [17] (*empty circles*; the ionic strength is 0.1 M, the molecular weight of the complexes is corrected, and the Φ value is equal to $0.9\times10^{23}$ obtained from Eq. 5). Curve *B*: SDS–protein complex of 1.4/1 binding level (*filled triangles*; the molecular weight of the complex is uncorrected and the Φ value is equal to $2.6\times10^{23}$)

In this concentration, the SDS–protein complex is found at the 1.4/1 binding level.

In our presentation, we use for the native proteins the Φ values obtained for each protein [21, 22] after a correction based on their deviation from the polystyrene calibration curve obtained in the same columns [23]. The intrinsic viscosity values of the native proteins are from an article of Tanford [15]. For the complexes, the value of Φ was calculated according to Eq. 5; the molecular masses are calculated by taking into account the SDS adsorbed, as before; and the intrinsic viscosity values are from the work of Reynolds and Tanford [1] as for the application of Eq. 4. The results obtained, with the complexes and the native proteins, show that all the points lie on the same curve (curve A, Fig. 2), suggesting an approvement of our proposal to take into account the SDS adsorbed in the determination of the molecular weight of the SDS–protein complex and of the determination of Φ by means of Eq. 5.

If, on the contrary, by applying the "modified universal calibration", we use for the complexes the molecular weight of the denaturised proteins obtained in GuHCl and for Φ, the value $2.6\times10^{23}$, we obtain curve B in Fig. 2.

Mori and Barth [20] have studied also by GPC the SDS–protein complexes in 0.1 M ionic strength and 3.5 mM SDS concentration. In these conditions, Rao and Takagi [17] have studied viscometrically some SDS–protein complexes. With these viscometric results and applying the "modified universal calibration", we have obtained the points in Fig. 2 shown by open circles. This result confirms again the proposed procedure of treating SDS–protein complexes as wormlike polymers.

We must indicate that we have not used the viscometric results obtained with the low molecular mass complexes given in [1] because in the GPC study by Mori and Barth [20] (log$M$ versus elution volume in 0.02 M ionic strength), they are eluted in the same elution volume. This "anomalous" behaviour in the GPC curve is not observed in the curve obtained by the same authors, in 0.1 M ionic strength.

## Conclusion

We have obtained the unperturbed dimensions parameter $K_\Theta$ and the Kuhn statistical segment length of the complexes SDS–protein in the two binding levels, 1.4/1 and 0.4/1. From the $K_\Theta$ value obtained using Eq. 4, we calculated the Kuhn statistical segment length, $A$, of these complexes by means of Eq. 3. The value of Φ was obtained from Eq. 5. The statistical segment length of the complex 1.4/1 was found equal to 76 Å, longer enough than that of the 0.4/1 complex, which was found equal to 30 Å. This difference can be attributed to the higher charge of the 1.4/1 complex in comparison with that of the 0.4/1 complex not only because of its higher composition in SDS but also the ionic strength of the solution seems to further support this behaviour.

At this point, we should emphasise that even the stiffer complex, 1.4/1, presents a random coil behaviour due to the relatively high number of its statistical segments, about 15, contained even in the lower molecular weight protein sample used, 35,000. Finally, our study suggests that the SDS–protein complexes could be assimilated with wormlike polymers because of the high value of their statistical segment length. As a result, they present an important draining effect, and only if we take into account the influence of this draining effect on the Φ value (Eq. 5), then we obtain a correct value for the hydrodynamic volume of the complex in solution as it is demonstrated by the GPC study.

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