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Calorimetric and viscosimetric investigation of the interaction between α,β-polyasparthydrazide and sodium dodecyl sulfate micelles

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Summary

The interaction between α,β-polyasparthydrazide (PAHy) and sodium dodecyl sulfate (SDS) micelles in aqueous solution was investigated by calorimetry and viscosimetry. The dependence of the enthalpic effect due to this interaction upon the surfactant concentration was rationalized in terms of a progressive binding of SDS micelles to the polymeric backbone. The analysis of the calorimetric data allow evaluation of the binding ability of SDS micelles to the polymeric chain. The viscosimetric behavior of SDS plus PAHy aqueous solutions, discussed in terms of the parameter F [$F = \eta_{rel(PAHy)} + \eta_{rel(SDS)} - \eta_{rel(SDS+PAHy)}$], confirms the occurrence of the interaction between SDS micelles and the PAHy macromolecule.

Introduction

α,β-Polyasparthydrazide (PAHy) is a water-soluble polymer which was synthesized in our laboratory some years ago (Giammona et al., 1987, 1989). It has interesting properties suggesting its possible use in the pharmaceutical field as a plasma expander or drug carrier. In particular, it was previously shown (Giammona et al., 1993) that the injection of PAHy solutions in laboratory animals does not induce acute and subacute toxicity and that no significant variation of the haematological and haematic parameters of laboratory animals is observed. In addition, PAHy aqueous solutions do not markedly change the arterial systolic and diastolic pressure and the rate of heart beat of unbled animals within the 2 h following administration. On the other hand, the same PAHy aqueous solutions administered to bled animals immediately restore the normal haematic parameters. These effects remain unchanged throughout the 2 h following bleeding.

From a structural point of view, the PAHy macromolecules have several terminal reactive hydrazine groups which enable them to link readily, by chemical bonds, drug molecules bearing carboxylic or aldehydic groups. These drug-PAHy

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conjugates are potentially advantageous therapeutic agents since they could minimize the side effects due to drug hyperdosage and could prolong drug delivery. Moreover, the biostability, biosolubility and pharmacokinetcs of drugs can be strongly enhanced by their attachment to the hydrophilic polymer.

Unfortunately, no knowledge on the physicochemical properties of this polymer and on its binding ability to biomembranes is available despite being of great importance in order to rationalize the efficiency of drug-PAHy conjugates in biological environments. However, it should be stressed that many difficulties arise in the interpretation of experimental data concerning the interaction between polymers and cellular systems (due to the several processes occurring during the experiments in cells). In order to surmount these difficulties, it has been suggested to use micellar aggregates as biomembrane models (Giammona et al., 1990, 1992; Cavallaro et al., 1993a). This is because micellar aggregates share some fundamental features of biomembranes such as the dominance of interfacial effects on their behavior and the existence of a nanoscopic apolar interior formed by an ordered array of molecules (Fendler, 1987).

According to these considerations, we have previously studied the interaction between α,β-poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) (Neri et al., 1973; Antoni et al., 1974), and surfactant micelles (Giammona et al., 1990; Carlisi et al., 1992; Cavallaro et al., 1993a,b). Our investigations have demonstrated that PHEA, a polymer structurally similar to PAHy, interacts with surfactant micelles forming a PHEA-micelle complex. In addition, PHEA interacts more strongly with anionic than with cationic or non-ionic surfactants. Finally, it was noted that electrostactic and hydrophobic effects both contribute to the PHEA-micelle interaction.

In order to ascertain whether a similar complex is formed between PAHy and micelles and to obtain specific information on its properties, we have undertaken a calorimetric and viscosimetric investigation on the interaction between PAHy and SDS micelles in aqueous solutions as a function of surfactant concentration. The aim of this paper is to report and discuss these experimental results and to compare the behavior of PAHy-SDS micelle complex with that formed by PHEA and SDS micelles.

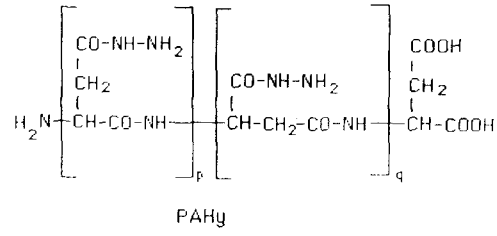
Materials and Methods

Synthesis and purification of PAHy were performed as previously reported (Giammona et al., 1987, 1989). The structure of PAHy is shown in Fig. 1.

Sodium dodecyl sulfate (SDS, Sigma product with 99% of stated purity) was used without further purification. Water was deionized and double distilled.

Calorimetric measurements were performed at 25°C with an LKB Thermal Activity Monitor (TAM) equipped with a flow-mix cylinder (LKB, 2277–204). The solutions were driven by a peristaltic pump (Gilson, Minipuls 2) and the flow rates (nearly 0.004 g s^{-1}) were determined by mass. Each calorimetric measurement was carried out by mixing inside the calorimetric cell an aqueous solution at fixed PAHy molonity (mol of solute per kg of solution = 6.8×10^{-5} mol kg^{-1}) with an aqueous surfactant solution. The thermal effect arising from the dilution of the SDS aqueous solution was subtracted taking as baseline the signal obtained by mixing into the calorimeter this solution with water. No thermal effect due to the mixing of the PAHy solution with water was observed. The experimental molar enthalpy (ΔH_{exp}) was calculated per mol of polymer.

The viscosities at 25 ± 0.01°C were measured with an Ubbelohde micro-viscosimeter equipped



PAHy

Fig. 1. Structure of PAHy (*p* and *q* denote the numbers of repetitive units constituting the polymeric backbone).

with an AVS 440 automatic viscosity measuring unit from Schott. The micro-viscosimeter was chosen with a sufficently long time ($t > 100$ s) in order to minimize the kinetic energy correction. The calibration procedure was described previously (D'Aprano et al., 1990).

The PAHy concentration of the aqueous solutions with and without SDS was constant (0.422% w/w). The densities required to convert kinematic viscosities to dynamic viscosities (η) were taken from the literature (De Lisi et al., 1984).

Results and Discussion

The experimental molar enthalpies (ΔH_{exp}) due to the interaction between PAHy and SDS in aqueous solutions as a function of the surfactant molonity [SDS], are reported in Table 1 and graphically depicted in Fig. 2. For comparison, Fig. 2 also illustrates the trend of ΔH_{exp} of interaction between PHEA and SDS previously reported (dashed line) (Carlisi et al., 1992).

TABLE 1

Molar enthalpy (ΔH_{exp}, kJ mol^{-1}) of interaction between PAHy and SDS as a function of SDS molonity

[SDS]	ΔH_{exp}
0.00315	−2.51
0.00385	−7.08
0.00537	−16.1
0.01186	−85.4
0.02548	−93.3
0.03421	−128.6
0.06261	−152.0
0.1093	−176.2
0.1321	−182.6
0.1764	−191.7
0.1985	−175.8
0.2647	−175.0
0.3152	−170.4
0.3658	−192.9
0.3989	−174.2
0.4177	−175.0
0.4234	−192.6

As can be seen, in the range $0 < [SDS] < 0.005$, for both polymers only small thermal effects occur (see inset to Fig. 2). Considering that the

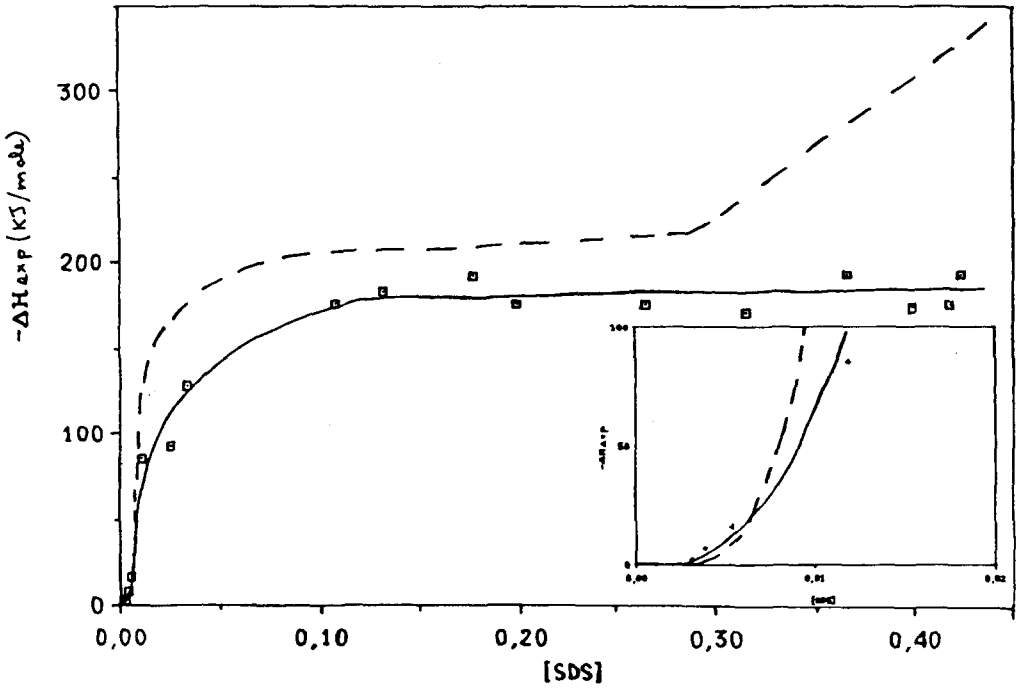


Fig. 2. Molar enthalpy (ΔH_{exp}, kJ mol^{-1}) of the interaction between PAHy and SDS as a function of [SDS]. The dashed line represents the trend of ΔH_{exp} for PHEA.

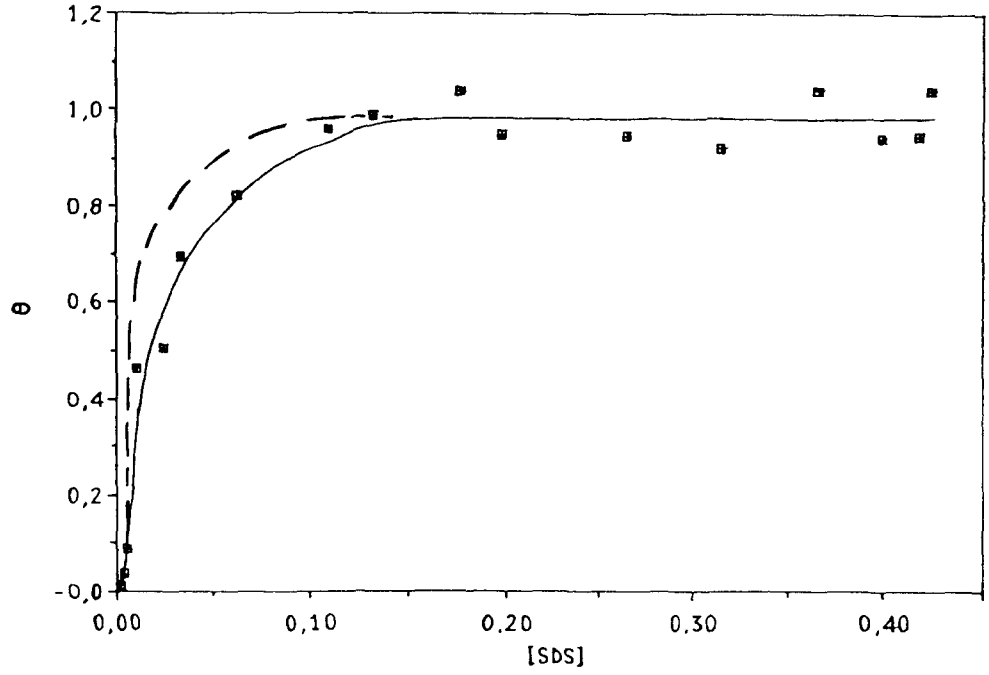


Fig. 3. Fraction of binding sites occupied (θ) by the micelles as a function of [SDS] for the PAHy macromolecule. The dashed line represents the trend of θ for PHEA.

critical micellar concentration (CMC) of SDS in water is 8.3×10^{-3} M, that the presence of the polymeric molecules induces a lowering of the CMC and that, below the CMC, the surfactant molecules are essentially in the monomeric state, the observed small thermal effects can be attributed to the interaction between the polymeric molecules and the small fraction of surfactant in the micellized state in equilibrium with the monomers.

In the range $0.005 < [\mathrm{SDS}] < 0.25$, for both polymers, a rapid increase in ΔH_{exp} followed by a plateau region is observed. This behavior has been explained in terms of a progressive binding of micellar aggregates to the polymer molecules. In accord with this interpretation, the plateau represents the region where the polymeric chains are totally saturated. In order to have a more quantitative description of the binding ability of both polymers, let us assume that each polymeric chain has N binding sites, identical and independent. It follows that the fraction of sites (θ) occupied by the micelles is related to the concentration $[\mathrm{Sn}]_b$ of the bonded micelles by the equation:

$$\theta = [\mathrm{Sn}]_b/(N \cdot [\mathrm{P}]) \quad (1)$$

where [P] is the molonity of the polymer. On the other hand, since:

$$\Delta H_{exp} = n \cdot \Delta H_M \quad (2)$$

where n is the number of binding sites of each polymeric chain occupied by micelles and ΔH_M denotes the molar binding enthalpy and, since:

$$\Delta H_b = N \cdot \Delta H_M \quad (3)$$

where ΔH_b is the enthalpy change accompanying the complete saturation of 1 mol of polymeric molecules (i.e., the value of ΔH_{exp} at the plateau), it follows that:

$$\theta = \Delta H_{exp}/\Delta H_b \quad (4)$$

The θ values, calculated from Eqn 4, are shown as a function of the surfactant concentration in Fig. 3. For comparison, Fig. 3 also depicts the analogous trend of θ for PHEA (dashed line) (Carlisi et al., 1992).

Comparison of the two trends suggests that the PHEA-SDS micelle complex is more stable than the PAHy-SDS micelle complex.

Finally, as shown in Fig. 2, at [SDS] > 0.25, only the ΔH_{exp} of PHEA increases with increasing [SDS]. This behavior can be reasonably explained by assuming that at [SDS] > 0.25 the SDS micelles are able to induce conformational changes of the polymeric chain of PHEA (involving an increase in sites accessible to the micelles) whereas this does not occur for PAHy at least until about [SDS] = 0.45.

Let us now consider the viscosimetric behavior. The dynamic viscosity values of SDS and SDS plus PAHy aqueous solutions as a function of [SDS] are collected in Table 2 whereas Fig. 4 shows the relative viscosities (η/η_0, where η and η_0 are the viscosity of the solution and of water, respectively) of the aqueous solutions of SDS and SDS plus PAHy as a function of SDS molonity. For comparison, Fig. 4 demonstrates the trend of η/η_0 of SDS plus PHEA aqueous solutions (dashed line) (Cavallaro et al., 1993a).

TABLE 2

Dynamic viscosities (η, cP) of SDS and SDS plus PAHy aqueous solutions as a function of the surfactant molonity at 25°C

SDS		SDS + PAHy	
[SDS]	η	[SDS]	η
0.00000	0.890	0.00000	0.917
0.00036	0.889	0.00246	0.923
0.00106	0.891	0.00697	0.915
0.00234	0.891	0.01490	0.928
0.00649	0.894	0.0346	0.989
0.02533	0.943	0.0534	1.057
0.1265	1.212	0.1247	1.239
0.2527	1.569	0.1883	1.423
0.2908	1.730	0.2604	1.660
0.4989	2.680	0.3684	2.101
0.5888	3.286	0.4880	2.703
		0.5846	3.391

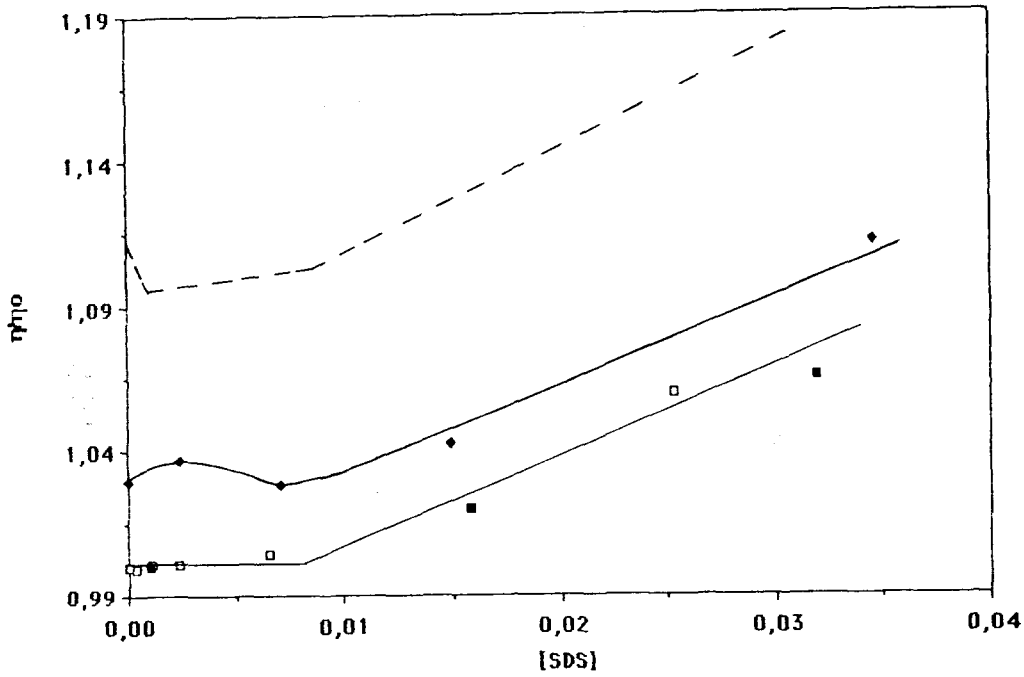


Fig. 4. Relative viscosities (η/η_0) of SDS (□, this paper; ■, Cavallaro et al., 1993a) and SDS plus PAHy aqueous solutions (♦) as a function of [SDS]. The dashed line represents the trend of η/η_0 for the SDS plus PHEA aqueous solutions.

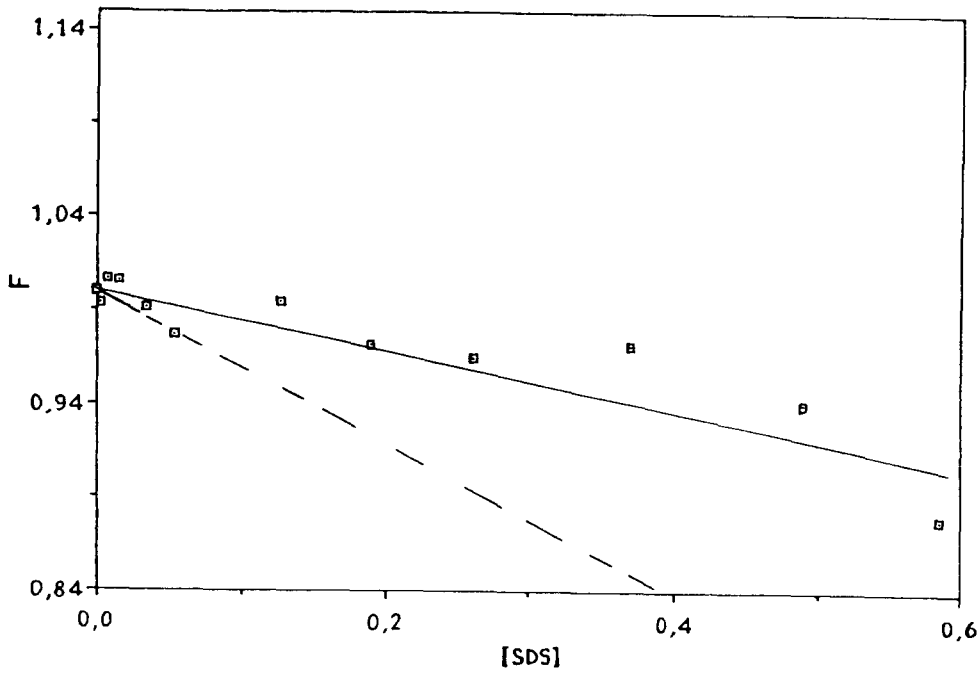


Fig. 5. F parameter (see text) as a function of [SDS] for PAHy. The dashed line represents the trend of the F parameter for PHEA.

As can be seen, the relative viscosity of SDS aqueous solutions starts to increase at a surfactant concentration quite close to the CMC of SDS and this increase has been attributed to the presence of micelles at [SDS] > CMC. When PAHy or PHEA are present in the system, it can be noted that at [SDS] < CMC the relative viscosity is not constant. According to Cohen and Priel (1990), this behavior can be attributed to the screening of long-range electrostatic interactions between the polymeric molecules due to the ions of the surfactant (Jon and Chang, 1990). At [SDS] > CMC (when micelles are present in the system), the relative viscosity increases with increasing surfactant concentration. According to Lewis and Robinson (1970), if interaction between SDS and polymer is absent, it can be expected that:

$$\eta_{(SDS+PAHy)} = \eta_{(PAHy)} + \eta_{(SDS)} - \eta_{(water)} \qquad (5)$$

and hence:

$$\eta_{rel(PAHy)} + \eta_{rel(SDS)} - \eta_{rel(SDS+PAHy)} = 1 \qquad (6)$$

Therefore, the deviation from unity of the quantity F

$$F = \eta_{rel(PAHy)} + \eta_{rel(SDS)} - \eta_{rel(SDS+PAHy)} \qquad (7)$$

can be taken as a measure of the interaction between the polymer and SDS. In Fig. 5, the F values calculated for PAHy as a function of [SDS] are shown. In addition, in Fig. 5 the dashed line represents the trend of F for PHEA.

As can be seen, F decreases with increasing [SDS], demonstrating the occurrence of PAHy-SDS micelle interaction. According to the above reported interpretation of the calorimetric data, this interaction can be explained by supposing that a progressive binding of micelles to the polymeric backbone occurs (Fishman and Eirich, 1975).

Further effects involving a decrease in F can derive from long-range interaction between free micelles and polymer-micelle complex. It can be also noted (see Fig. 5) that the slope of the F vs [SDS] plot of PAHy is less negative than that of

PHEA. This, according to the calorimetric results, suggests that the interaction of SDS micelles with PHEA is stronger than that with PAHy. In conclusion, given the similarity between SDS micelles and cellular membranes, it can be expected that the binding ability of PAHy to the cellular membranes is lower than that of PHEA.

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