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Influence of the albumin concentration and temperature on the lysis of human erythrocytes by sodium dodecyl sulfate

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Abstract The stability of human erythrocytes to sodium dodecyl sulfate (SDS) was assessed spectrophotometrically in the presence of different concentrations of bovine serum albumin (BSA) and at different temperatures (27–45 °C). The absorbance at 540 nm (A_{540}) was correlated with the SDS concentration by sigmoidal regression based on the Boltzmann equation. Erythrocyte stability was characterized on the basis of the SDS concentration that induces hemolysis in 50% of the cells (D_{50}). Progressive increases in the albumin concentration led to increases in the D_{50} value. The protective effect of BSA against SDS-induced hemolysis was attributed to the binding of the surfactant to the hydrophobic binding sites of this protein. The D_{50} values decreased sigmoidally with an increase in the temperature. This trend, which could not be explained by changes in the spectral properties of hemoglobin, maybe due to heterogeneity in the erythrocyte population.

Keywords Albumin · Erythrocytes · Osmotic stability · Sodium dodecyl sulfate · Surfactants · Temperature

Introduction

Surfactants or detergents are amphiphilic compounds that can be classified as nonionic, cationic, anionic, or amphoteric, depending on the nature of their polar group (Bhairi 2001).

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Sodium dodecyl sulfate (SDS; chemical formula CH_3-$(CH_2)_{11}$-$OSO_3^-Na^+$) is a well-known anionic detergent. It is widely used in the pharmaceutical industry in the production of cosmetics and in the chemical industry to increase the absorption of herbicides such as atrazine (Sanchez-Camazano et al. 2000). SDS is used to denature proteins (Liu et al. 2007; Moosavi-Movahedi 2005; Tanford 1968) and solubilize membranes (Helenius and Simons 1975).

Lysis of erythrocytes by SDS was studied by Sagit Shalel (Shalel et al. 2002). It is believed that in saline solutions, SDS acts on erythrocytes by solubilizing the membrane and not by osmotic lysis. There is a predominance of negative charges on the outer side of the erythrocyte membrane. In saline, there is a reduction of the negative charges on the membrane surface, which favors the aggregation of SDS molecules such that the effect of hemolysis is no longer an osmotic effect but is instead a solubilization effect. Hemolysis was reported to occur at SDS concentrations of approximately 104 μM (Bielawski 1990).

The plasma has high concentrations of albumin, a protein important in the binding and transport of hydrophobic compounds such as fatty acids, unconjugated bilirubin (Murray et al. 2003; Zunszain et al. 2003), drugs (Kandagal et al. 2006), and toxic chemical agents (Li et al. 2008).

Albumin can also bind to SDS and other amphiphilic substances, and this would certainly affect the amount of chaotrope available for promoting hemolysis.

In this study, we investigated the effect of the albumin concentration on the lysis of human erythrocytes by SDS. The protocols used here have been described earlier in literature (Cunha et al. 2007; Penha-Silva et al. 2008). A more thorough characterization of this system is of great importance because the stability of the erythrocyte mem-

brane has been extensively studied in relation to aging and longevity (Penha-Silva et al. 2007), degenerative diseases (de Freitas et al. 2010; Gilca et al. 2008; Mansur et al. 2010), action of natural products (de Freitas et al. 2008), drugs (de Jong et al. 2006; Rabini et al. 1993), and toxic agents (Narendra et al. 2007; Karabulut et al. 2009).

Since heat is also a chaotropic agent for proteins (Fonseca et al. 2006) and cells (Cunha et al. 2007; Penha-Silva et al. 2008; Tsong and Kingsley 1975), it can influence the chaotropic effect of SDS (Lee et al. 1989). The influence of temperature on erythrocyte lysis by SDS is also investigated in this study.

Materials and methods

The design of this study was approved by the local institutional ethics committee. The experiments were conducted with the understanding and consent of the volunteers.

Blood sample collection

Blood samples (3 ml) were collected from 8 female human volunteers (20–25 y, healthy, nonsmokers, nondrug addicts, and nonregular consumers of alcoholic beverages) by intravenous puncture after nocturnal fasting (8–14 h). Blood samples were collected in evacuated tubes containing 50 μl of heparin (Vacutainer, Becton Dickinson, Juiz de Fora, MG, Brazil).

Reagents and equipments

The NaCl used (Synth, São Paulo, SP, Brazil) was 99.5% pure, which was taken into consideration during solution preparation. The bovine serum albumin (BSA) used was 98% pure (Sigma, St. Louis, MO, USA). Human hemoglobin was obtained from a concentrate of erythrocytes by lysing the cells against distilled water. Volume measurements were carried out with refractory glass burettes or automatic pipettes (Labsystems, Helsinki, Finland). Mass measurements were recorded on a digital balance (AND, Japan). Samples were incubated in a refrigerated water bath (Marconi, Piracicaba, SP, Brazil). Absorbance readings and spectral scans were performed with a digital spectrophotometer (model UV-1650PC, Shimadzu, Kyoto, Japan).

Determination of the stability of erythrocytes in physiological saline solution at increasing SDS concentrations

SDS solutions prepared in 0.9 gdl^{-1} NaCl (saline) and ranging in concentration from 93.6 to 191 μM were used to analyze the stability of erythrocytes in the absence of added albumin. The effects of the albumin concentration (0.02, 0.04, 0.06, 0.08, or 0.1 gdl^{-1}) were examined using saline SDS solutions ranging in concentration from 93.6 to 274 μM.

A series of test tubes (Eppendorf, Brazil) was prepared in duplicate with 1 ml of the test solution, and these were preincubated for 10 min. After adding 10 μl of blood to the solute, the solution was homogenized and then incubated at 37 °C for 30 min. After centrifugation for 10 min at 2,000×*g*, the supernatant was separated and analyzed by visible (VIS) spectrophotometry at 540 nm.

Determination of the UV–VIS spectra of hemoglobin at different SDS concentrations

Spectral scans were performed between 250 and 700 nm using samples of human hemoglobin and whole blood in saline solutions (0.9 gdl^{-1} NaCl) of SDS at concentrations before (101 and 107 μM) and after the sigmoidal transition of hemolysis (177 and 184 μM).

Determination of hemolysis transition curves

The dependence of the values of A_{540} on the SDS concentration was adjusted by a sigmoidal regression line given by the Boltzmann equation

$$A_{540} = \frac{A_1 - A_2}{1 + e^{(D - D_{50})/dD}} + A_2, \quad (1)$$

in which A_1 and A_2 represent the minimum and maximum values of hemolysis, D is the SDS concentration, D_{50} represents the SDS concentration at which 50% of the cells undergo hemolysis, and dD is the variation in the SDS concentration during the sigmoidal transition between A_1 and A_2.

Statistical analyses

Calculations and statistical analyses were performed with the OriginPro 8.0 (Microcal, Northampton, MA, USA) software package. The regression lines were considered significant only when p was less than 0.05.

Results

The effect of BSA on the lysis of human erythrocytes as a function of the SDS concentration in 0.9 gdl^{-1} NaCl is shown in Fig. 1. In the absence of BSA (Fig. 1a), full hemolytic transition was observed between 0 and 190.7 mM SDS. Addition of BSA at concentrations of 0.02 (Fig. 1b), 0.04 (Fig. 1c), and 0.06 (Fig. 1d) g dl^{-1} resulted in a right shift of the transition curve of lysis, with an accompanying increase in the point of half-transition

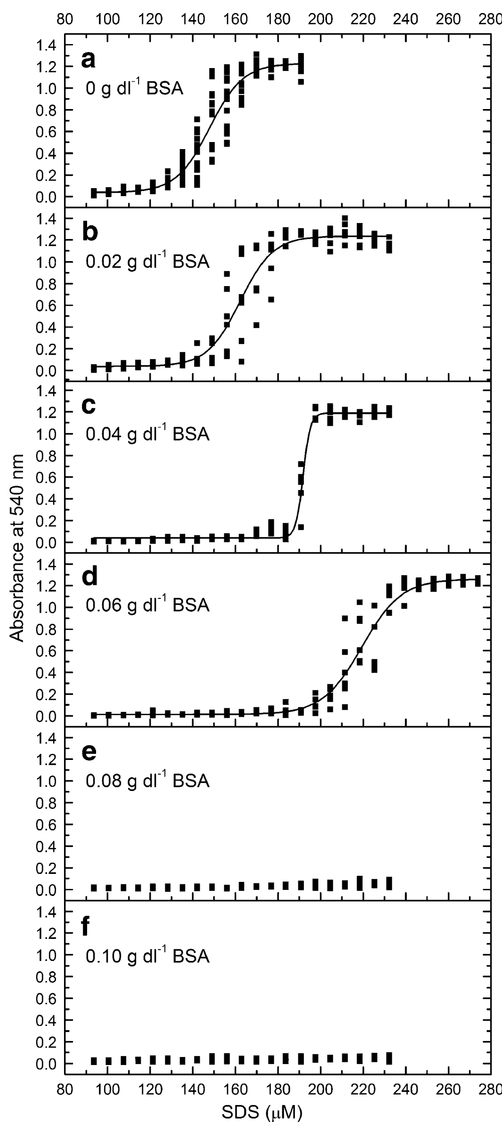

Fig. 1 Influence of the BSA concentration on SDS-induced hemolysis. Condition: 0.9 gdl^{-1} NaCl

(D_{50}) of the hemolysis. At 0.08 (Fig. 1e) and 0.10 gdl^{-1} (Fig. 1f) BSA, the surfactant did not produce hemolysis in the concentration range studied.

This effect of BSA on SDS-induced hemolysis does not appear to be an artifact arising from the influence of SDS on the spectral properties of BSA because the UV–VIS BSA spectra was not significantly perturbed at the SDS concentrations used to produce full hemolytic transition (data not shown).

As a chaotrope, heat is believed to potentiate SDS-induced hemolysis. The influence of temperature on SDS-induced hemolysis is shown in Fig. 2. An increase in

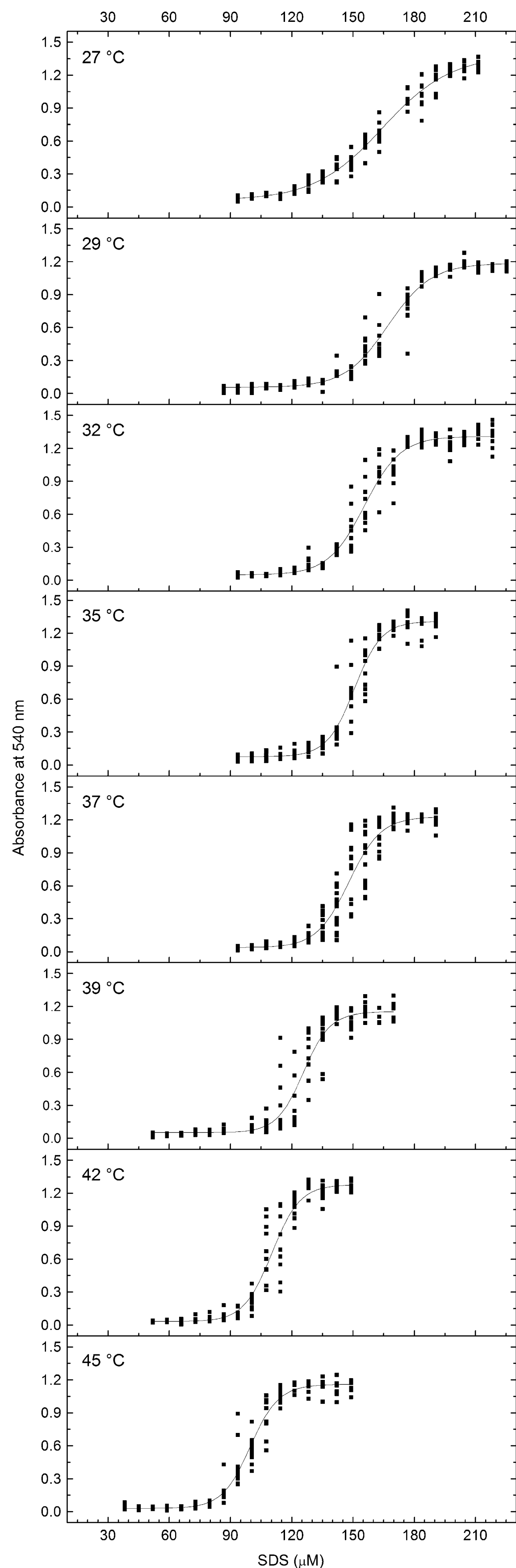

Fig. 2 Influence of the temperature on SDS-induced hemolysis. Condition: 0.9 gdl^{-1} NaCl

temperature from 27 to 45 °C is associated with a left shift of the curves of SDS-mediated hemolysis, with an accompanying decrease in the D_{50} values.

The effect of temperature on the D_{50} values associated with SDS-induced hemolysis is shown in Fig. 3. The dependence of D_{50} on the temperature is best described by a decreasing sigmoid curve.

This effect maybe due to the influence of the temperature increase on the spectral properties of hemoglobin. To examine this possibility, UV–VIS hemoglobin spectra were recorded in the same temperature range (27–45 °C) (data not shown). Under the experimental conditions, the effects of hemolytic concentrations of SDS on the A_{540} values of hemoglobin were significantly associated with a decreasing regression line of very small slope (0.000474).

Discussion

Erythrocytes are an excellent model for studying membrane stability (Cunha et al. 2007; de Freitas et al. 2008; de Freitas et al. 2010; Penha-Silva et al. 2008; Penha-Silva et al. 2007; Mansur et al. 2010) due to their easy availability and low cost.

The membrane stability of erythrocytes is estimated in terms of the concentration of denaturing agent required to hemolyze 50% of the cells. In this analysis, the absorbance of the hemoglobin released during the rupture of the erythrocyte membrane was recorded at 540 nm.

The hemolysis curve is characterized by a two-state transition (Fig. 1). In the absence of added BSA (Fig. 1a), lysis begins at approximately 130 μM SDS and is completed around 160 μM SDS. The SDS concentrations that define the first and second plateaus of the sigmoid represent the regions of minimal and maximal hemoglobin release, respectively.

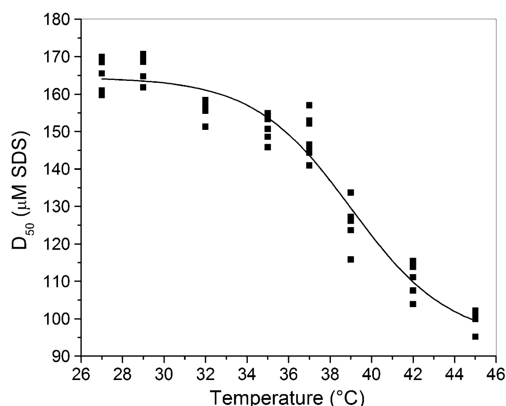

Fig. 3 Dependence of the temperature of the half-transition point (D_{50}) during SDS-induced hemolysis. Condition: 0.9 gdl^{-1} NaCl

Since SDS is a chaotrope and can denature proteins such as hemoglobin, we investigated the effect of hemolytic concentrations of SDS on the UV–VIS spectral properties of these proteins. SDS produced only slight changes in the intensity of the human hemoglobin bands. The SDS concentrations required to promote hemolysis do not perturb the hemoglobin spectra. In fact, conformational changes in hemoglobin with spectral perturbations were described at much higher SDS concentrations (above 69 mM) (Mitjans et al. 2008).

At lower concentrations, SDS treatment only results in hemolysis (Bielawski 1990). In fact, 50% hemolysis was observed at 148 μM SDS and 37 °C in the absence of added BSA (Fig. 1a). The conditions under which this experiment was performed do not reflect the albumin concentration of the blood because the blood sample (10 μl) was diluted in a saline solution (0.9 gdl^{-1}) of SDS (1,000 μl). Albumin is present in the blood at concentrations between 3.5 and 5.5 gdl^{-1} (Murray et al. 2003). Incorporation of 0.02 to 0.06 gdl^{-1} BSA in the reaction medium resulted in a right shift of the hemolysis curve of SDS (Fig. 1b to d), with an increase in the D_{50} values. This indicates that BSA can protect erythrocytes against lysis by SDS. Albumin can bind to several compounds of endogenous (Zunszain et al. 2003) and exogenous (Kandagal et al. 2006; Li et al. 2008) origins, including SDS (Kragh-Hansen et al. 2001). The stabilizing effect of albumin is probably due to its binding to SDS, which is expected to decrease the availability of the detergent and thereby obstruct micelle formation. An increase in the BSA concentration from 0 to 0.06 gdl^{-1} (Fig. 1b to d) decreased the cooperative effect of detergent molecules in the lysis of erythrocytes. At BSA concentrations higher than 0.06 gdl^{-1} (Fig. 1e to f), the availability of SDS for erythrocyte lysis decreased such that no hemolysis was observed in the detergent concentration range studied. Certainly, the increasing albumin concentration resulted in increased availability of detergent-binding sites, which decreased the bioavailability of SDS for promoting hemolysis. Thus, the albumin concentration is an important factor in this system.

Temperature is another variable that needs to be controlled in such experiments. Temperature increase in the range of 27–45 °C caused a left shift in the SDS-induced hemolysis curves (Fig. 2). Since heat is also a chaotrope, temperature increase is expected to lead to a decrease in the D_{50} value for SDS, and this was actually observed. The dependence of D_{50} on the temperature was better fitted by a decreasing sigmoid (Fig. 3). The sigmoid nature of this fit is regarded to be a consequence of the changes in the spectral behavior of hemoglobin upon temperature increase because heat is also a chaotrope for proteins. In fact, there was a significant but slight decrease in the A_{540} values of hemoglobin as the temperature

increased. Subtraction of the effect of temperature on the A_{540} value of hemoglobin from the total A_{540} values associated with SDS-induced hemolysis curves at different temperatures only produced changes in the values of D_{50} at the centesimal level (data not shown). The negligible impact of the studied SDS range on the VIS spectral properties of hemoglobin indicated that the sigmoidal nature of the curve of D_{50} versus temperature (Fig. 3) could have some other specific physical meaning.

The sigmoidal decrease in the D_{50} values (Fig. 3) is probably indicative of the possibility that at lower temperatures of the studied temperature range, the synergistic effects of heat and SDS had a smaller impact on hemolysis (first plateau). With further temperature increase, the synergism is potentiated (intermediary descending region of the sigmoid). Beyond this region, the synergism becomes weaker (second plateau). This weakening of the action synergism between heat and SDS could be due to the heterogeneity in the erythrocyte population (Bielawski 1990; Jay and Rowlands 1975). The existence of erythrocytes that are more resistant to lysis may be the reason for the weakening of the chaotrope synergism of heat and SDS in the second plateau of the sigmoid. This heterogeneity may arise from the natural composition of the blood or from the stabilizing effect generated by the combination of heat and SDS. The existence of such a stabilizing effect implies the occurrence of action dualism in the system of SDS plus heat. Moreover, both these agents are chaotropes and can affect the osmotic pressure of the medium. In fact, shape changes (Vives et al. 1999) and antihemolytic changes (Sánchez et al. 2007) have been described for the interactions between certain surfactants and erythrocytes.

Conclusions

Thus, the addition of BSA was found to protect human erythrocytes against SDS-induced hemolysis. This effect is attributed to the binding of SDS by BSA. The half-transition point of the SDS-induced hemolysis curve exhibits a sigmoidal decreasing dependence with temperature increases between 27 and 45 °C. This effect was attributed to heterogeneity in the human erythrocyte population.

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