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Elucidating the mode of action of urea on mammalian serum albumins and protective effect of sodium dodecyl sulfate

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ABSTRACT

The effect of sodium dodecyl sulfate (SDS) on human, bovine, porcine, rabbit and sheep serum albumins were investigated at pH 3.5 by using various spectroscopic techniques like circular dichroism (CD), intrinsic fluorescence and dynamic light scattering (DLS). In the presence of 4.0 mM SDS the secondary structure of all the albumins were not affected as measured by CD but fluorescence spectra revealed 8.0 nm blue shift in emission maxima. We further checked the stability of albumins in the absence and presence of 4.0 mM SDS by urea and temperature at pH 3.5. In the absence of SDS, urea starts unfolding both secondary as well as tertiary structural elements of the all the albumins at ~2.0 M urea but in the presence of 4.0 mM SDS, urea was unable to unfold even up to 9.0 M. The albumins were thermally less stable at pH 3.5 with decrease in T_m but in the presence of 4.0 mM SDS, the T_m was increased. From this study, it was concluded that SDS is showing a protective effect against urea as well as thermal denaturation of albumins. This behavior may be due to electrostatic as well as the hydrophobic interaction of SDS with albumins. Further, we have proposed the mechanism of action of urea. It was found that urea interacted with proteins directly when proteins are in charged form. Indirect interaction may be taking place when the environment is more hydrophobic.

1. Introduction

Serum albumins play an important role in transportation and distribution of exogenous and endogenous ligands in the blood also maintain the physiological pH and osmotic pressure of the blood [1]. Human serum albumin (HSA), bovine serum albumin (BSA), porcine serum albumin (PSA), sheep serum albumin (SSA) and rabbit serum albumin (RSA) are globular protein consisting of a single polypeptide chain [2]. The stability of serum albumins has been studied in many in vitro studies [3–6]. Urea is a chemical denaturant which is widely exploited for investigating the conformational stability of proteins [7,8]. The molecular mechanism of urea induced protein unfolding is still a controversial issue. There are two types of mechanism proposed on the basis of experimental and theoretical observations. The first is an indirect mechanism, which propose that the urea disrupt the water molecules and help in salvation of hydrophobic groups [9]. According to the second mechanism, the urea directly interacted with protein by electrostatic or van der Waals forces [10]. Vast literatures is available on thermal denaturation [11–13]. Various strategies have been proposed to achieve stabilization of proteins including chemical modification, protein engineering, use of surfactants and polyhydroxy compounds [14]. Using these methods, the half-life, water solubility is also increased and self-aggregation property of proteins is reduced [15,16]. Many additives are routinely used for the solubilization of inclusion bodies and surfactant is one of them. Sodium dodecyl sulfate (SDS) is the most repeatedly studied surfactant. It is well documented that SDS is used for both stabilization and destabilization of proteins [17]. Available literature reveals that SDS interacts with proteins via ionic as well as hydrophobic interactions [18–20]. It is also reported that SDS is having great capacity to unfold the proteins [21,22]. In this study we have taken five serum albumins from different sources and studied the effect of SDS at pH 3.5 on the conformation of albumins. Further, we have seen the effect of urea and temperature in the presence of 4.0 mM SDS. Other objectives of this work were to investigate the mechanism of urea action.

2. Materials and methods

2.1. Materials

Essentially fatty acid free human serum albumin (068K7538V), bovine serum albumin (110M166IV), porcine serum albumin (094K7636), sheep serum albumin (117K7540), rabbit serum albumin (117K7565), urea and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemicals Co. (St. Louis, Mo, USA). All other reagents used were of analytical grade.

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2.2. Protein concentration determination

The protein concentrations were determined spectrophotometrically using molar extinction coefficients; ε_M 35700 M^{-1} cm^{-1} (HSA), 43827 M^{-1} cm^{-1} (BSA), 43385 M^{-1} cm^{-1} (PSA and RSA) and 42925 M^{-1} cm^{-1} (SSA) at 280 nm on Perkin–Elmer (Lambda 25) double beam spectrophotometer.

2.3. pH measurements

pH measurements were carried out on Metler Tolado pH meter (seven easy S 20-K) using Exper "Pro3 in 1" type electrode. The least count of the pH meter was 0.01 pH unit.

2.4. Circular dichroism

CD measurements were performed by a Jasco spectropolarimeter (J-815), equipped with a Jasco Peltier-type temperature controller (PTC-424S/15). The instrument was calibrated with D-10-camphorsulfonic acid. The measurements were carried out at 25 °C. Far-UV CD spectra were collected with a protein concentration of 5.0 μM with 0.1 cm path length in the range of 200–250 nm. Each spectrum was the average of 2 scans. Prior the measurements all the samples were incubated over night.

2.5. Fluorescence measurements

Fluorescence measurements were performed on Hitachi spectrofluorometer (F-4500) equipped with a PC. The fluorescence spectra were collected at the 25 °C with a 1 cm path length cell. The intrinsic fluorescence spectra were recorded between 300 and 400 nm with excitation wavelength of 295 nm. The excitation and emission slit widths were set at 5 nm.

2.6. Dynamic light scattering

DLS measurements were performed with protein concentration of 15.0 μM using DynaPro-TC-04 dynamic light scattering equipment (Protein solutions, Wyatt Technology, Santa Barbara, CA) equipped with temperature-controlled microsampler. Measured size was presented as the average value of 50 runs. Data were analyzed by using Dynamics 6.10.0.10 software at optimized resolution. The mean hydrodynamic radius (R_h) and polydispersity (Pd) were estimated on the basis of an autocorrelation analysis of scattered light intensity based on the translational diffusion coefficient, by Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta D_W^{25^\circ C}}$$

where R_h is the mean hydrodynamic radius, k is the Boltzman's constant, T is the absolute temperature, η is the viscosity of water and D is the translational diffusion coefficient.

3. Results

3.1. Effect of urea and SDS on secondary structure of serum albumins

Far-UV CD spectroscopy is greatly used to observe the changes in secondary structure, conformation and stability of proteins in solutions [23,24]. Far-UV CD spectra of all the five albumins at pH 3.5 exhibited two minima, one at 208 nm and other at 222 nm which is characteristic of α-helical structure [25]. Urea induced secondary structural change has been frequently observed [6,26]. As shown in Fig. 1A, in the presence of 4.0 mM of SDS ellipticity of spectra in all the albumins were same as that of spectra at pH 3.5. We further checked the effect of urea on albumins in the absence and presence of 4.0 mM SDS at pH 3.5, for sake of clarity only 4 representative spectra are shown, i.e., at 0, 2.0, 8.0 and 9.0 M urea. Up to 2.0 M urea the ellipticity of all albumins did not change but beyond this concentration ellipticity decreased maximally at 9.0 M urea. However, in the presence of 4.0 mM of SDS, the ellipticity of spectra was not changed even up to 9.0 M urea. The Fig. 1B shows urea induced unfolding of serum albumins in the absence and presence of 4.0 mM of SDS as monitored the change in CD (mdeg) at 222 nm. Urea unfolds the serum albumins without SDS at pH 3.5 with C_m values 3.44, 2.28, 2.49, 2.58 and 3.41 M in an HSA, BSA, PSA, RSA and SSA, respectively. However in the presence of 4.0 mM SDS urea was unable to unfold albumins even at higher concentration. From CD measurements, it can be concluded that in the presence of 4.0 mM SDS the secondary structures of serum albumins were stable against urea denaturation even up to 9.0 M urea.

3.2. Intrinsic fluorescence measurements

Proteins contain three type of aromatic amino acid residues (Trp, Tyr and Phe) which may contribute to their intrinsic fluorescence but only tyrosine and tryptophan is used experimentally because their quantum yields is high enough to give a good fluorescence signal. In Fig. 2A, shows the fluorescence emission spectra of all albumins. The maximum emission of fluorescence intensity was observed at 333 nm (HSA), 332 nm (BSA), 336 nm (PSA), 338 nm (SSA) and 336 nm (RSA). It was reported that if wavelength maximum is in the range of 330–340 nm, the protein is well folded and tryptophan is buried in a hydrophobic core [27]. After addition of 4.0 mM SDS in all albumins incubated at pH 3.5, a drastic change in the fluorescence emission spectrum was noticed and wavelength maximum showed a blue shift on an average of 8 nm. We further, studied the effect of increasing concentration of urea on the albumins in the absence and presence of 4.0 mM of SDS. As shown in Fig. 2A, in the absence of SDS the fluorescence emission spectrum was slightly red shifting in the presence of 2.0 M urea and maximum red shift was seen at 9.0 M urea with decrease in fluorescence intensity. However in the presence of 4.0 mM SDS the emission maxima were very minutely shifted even in the presence of 9.0 M urea with slight increase in fluorescence intensity. The shift in wavelength maximum of five albumins in the absence and presence 4.0 mM SDS are plotted at varying concentration of urea as shown in Fig. 2B. Albumins incubated at pH 3.5 reveled a sigmoidal change in emission maxima with increasing concentration of urea but in the presence of 4.0 mM SDS no sigmoidal change was noticed even at 9.0 M urea.

3.3. Hydrodynamic radii measurements

DLS is used to characterize the hydrodynamic radii (R_h) of proteins as well as to detect the conformational change of proteins. The R_h of albumins at pH 7.4 were found to be 3.4 nm (HSA), 3.7 nm (BSA), 3.9 nm (PSA) and 3.4 nm (RSA, SSA). At pH 3.5, a slight increase in R_h were observed i.e., 4.0 nm (HSA), 4.5 nm (BSA), 4.4 nm (PSA), 4.0 nm (RSA) and 4.4 nm (SSA) probably due to the partial unfolding of protein under acidic condition. The addition of 4.0 mM SDS at pH 3.5 caused decrease in R_h of partially unfolded albumins to 3.2 nm (HSA), 3.1 nm (BSA), 3.6 nm (RSA) and 3.9 nm (PSA, SSA), respectively owing to compaction of structure which was induced by SDS. The effect of urea was also observed on albumins at pH 3.5. The urea unfolds the albumins in the absence of SDS and a hydrodynamic radii increase accordingly is shown in Table 1. However, in the presence of 4.0 mM SDS, urea was incapable of unfolding as the hydrodynamic radii did not

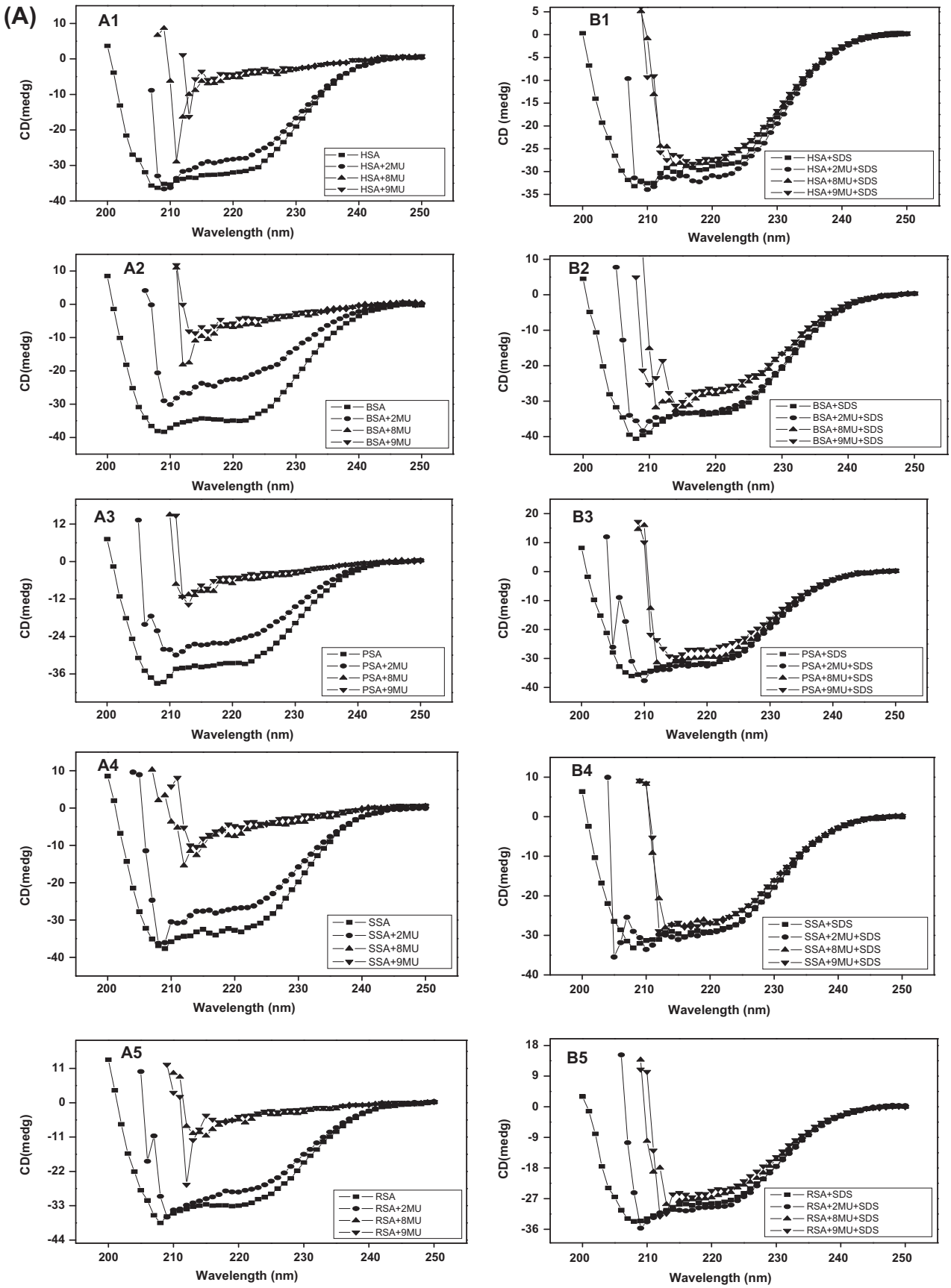

Fig. 1. The Far-UV CD spectra of albumins were monitored on the increasing concentration of urea in the absence (A) and presence (B) of 4.0 mM SDS on HSA, BSA, PSA, SSA and RSA at pH 3.5 (Fig. 1A). The overall helicity of (A) human, (B) bovine, (C) porcine, (D) sheep and (E) rabbit serum albumins at 222 nm in the absence and presence of 4.0 mM SDS as a function of varying urea concentration (Fig. 1B). In all the measurements albumin concentration was fixed 5.0 μM and all the samples were incubated overnight before the measurements.

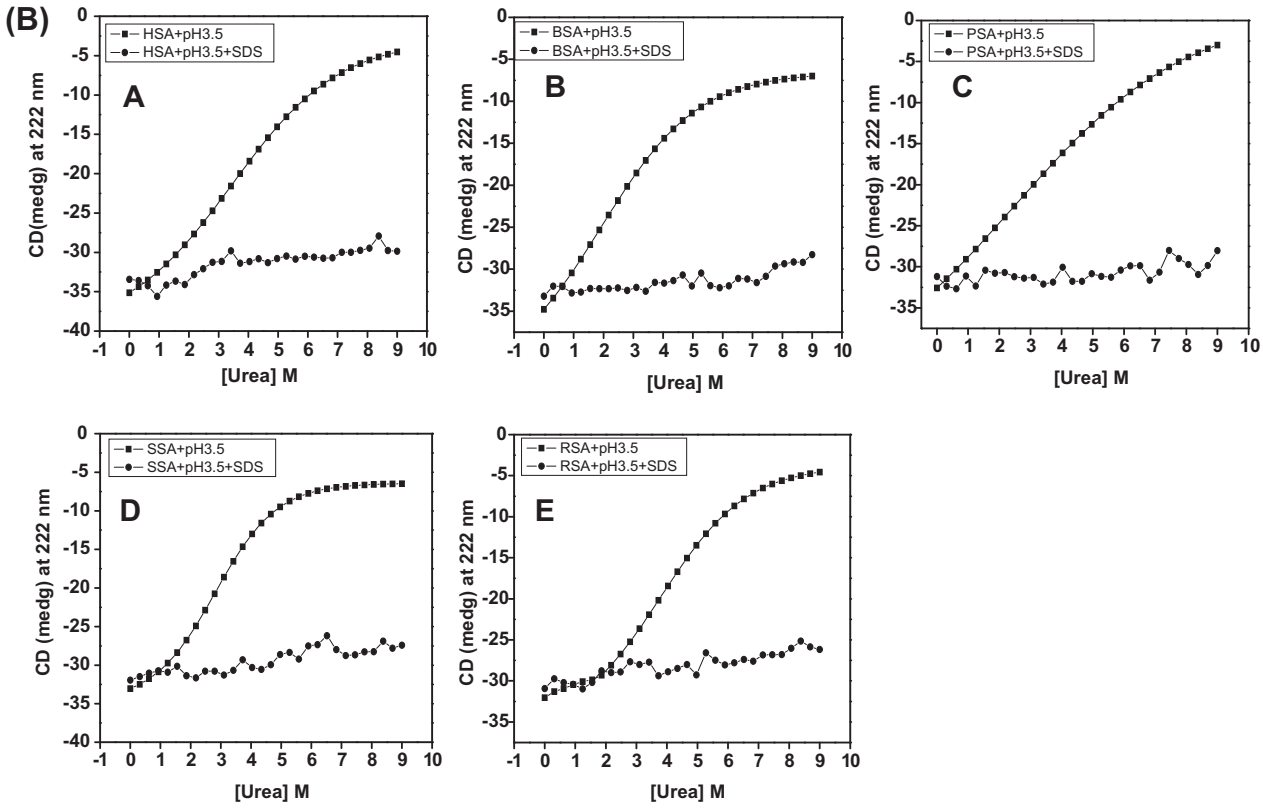

Fig. 1 (*continued*)

increase and it was more or less like native albumins. The hydrodynamic radii at different conditions are summarized in a Table 1.

3.4. Thermal denaturation

We also checked stabilizing effect of SDS on albumins against temperature changes. Fig. 3, summarizes the changes in CD ellipticity at 222 nm of native albumins and in the absence/presence of 4.0 mM SDS at pH 3.5. The native albumins (pH 7.4) show broad sigmoidal change with respect to temperature and the transition temperature (T_m) were 76 °C (HSA), 79 °C (BSA), 70 °C (PSA), 75 °C (SSA) and 70 °C (RSA) while at pH 3.5 the T_m was decreased significantly to 54 °C (HSA), 57 °C (BSA, PSA), 59 °C (SSA) and 63 °C (RSA) which suggest that secondary structure of albumins at pH 3.5 is very labile due to partial unfolding. We further incubated the albumins with 4.0 mM SDS at pH 3.5 and monitored the changes in secondary structure as a function of temperature. A sigmoidal change was found and T_m increased significantly to 74 °C (HSA), 75 °C (BSA), 70 °C (PSA, SSA) and 75 °C (RSA) which is close to native albumins. From the thermal denaturation profile it can be concluded that SDS is protecting the secondary structure of all albumins at pH 3.5.

4. Discussion

Various methods and additives are used for protein stabilization and denaturation [28,29]. In this study, we have seen the effect of SDS, urea and temperature on five different serum albumins at pH 3.5. It is reported that at neutral pH all the albumins exist in a compact form native or 'N' form while at low pH (pH 3.5) it is less compact i.e., 'F' form and this form comes due to conformational isomerization [30]. We observed the effect of SDS on the fast moving ('F') state of serum albumins. The secondary structures of fast moving serum albumins remained unchanged in the presence of 4.0 mM SDS while the tertiary structure shows some alteration with shift in wavelength maximum by 8.0 nm. The blue shifting behavior of proteins signifies that aromatic chromophores are shifting into the more non-polar environment. Similar results were also found in case of HSA where around 16 nm blue shift was noticed in the presence of 7.0 mM SDS at pH 7.0 [31,32]. We further explored the effect of urea and temperature on albumins conformation in the absence and presence of 4.0 mM SDS at pH 3.5. Urea unfolds the albumins in the absence of SDS at a little bit lower concentration (≃2.0 M) while in the presence of 4.0 mM SDS the urea is unable to unfold both secondary as well as tertiary structures of albumins even up to 9.0 M concentration. Urea unfold albumin at pH 7.4 and helicity of proteins is almost lost but at low concentration with the added SDS the helicity of proteins is again recovered which is very close to the native state of albumins. These results indicate that small amount of SDS refolded the unfolded protein [33]. Hydrodynamic radii of albumins were monitored by DLS. Albumins at pH 7.4 have compact structure and hydrodynamic radii are shown in Table 1 which is inconsistent with other reports [34]. At acidic pH, the hydrodynamic radii were found to increases due to partial unfolding. But in the presence of 4.0 mM of SDS the hydrodynamic radii of all albumins was decreased. The hydrodynamic radii of partially unfolded albumins were markedly increases in the presence of 9.0 M urea but in the presence of 4.0 mM SDS the urea is not capable of unfolding and changing the hydrodynamic radii as also supported our CD data. The protective behavior of SDS was also observed earlier and it is reported that SDS is wrapped around proteins with the help of a hydrophilic head and hydrophobic tail. This wrapping provides enough strength to proteins. At above CMC, SDS-protein interaction is based on two type of models one is a necklace and other is bead

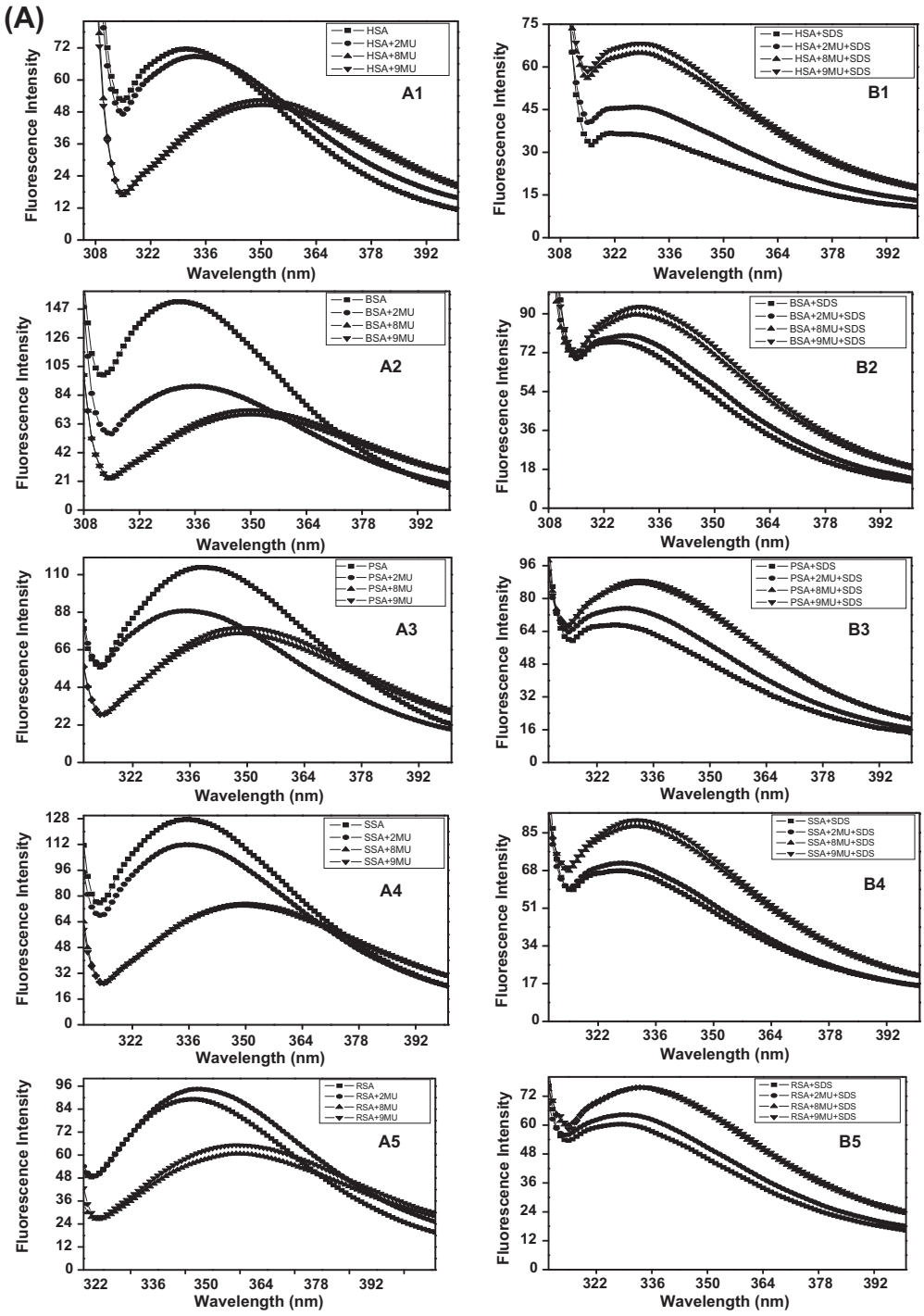

Fig. 2. Fluorescence emission spectra of (1) human, (2) bovine, (3) porcine, (4) sheep and (5) rabbit serum albumins at increasing concentration of urea in the (A) absence and (B) presence of 4.0 mM SDS at pH 3.5 (Fig. 2A). Change in wavelength maximum of HSA (-■-), BSA (-●-), PSA (-▲-), RSA (-▼-) and SSA (-♦-) in the (A) absence and (B) presence of 4.0 mM SDS with respect to increasing concentration of urea (Fig. 2B). The samples containing protein, SDS and urea were incubated overnight and protein concentration was 5.0 μM.

model. According to these two models the SDS is stabilizing the protein [35,36]. It is reported that urea has two types of denaturing property either directly or indirectly. From the above observation we can say that initially urea is favorably interacting with albumins electrostaticaly and through hydrogen bonding because albumins are positively charged at pH 3.5. But in the presence of 4.0 mM SDS at same pH the urea is unable to interact directly, the reason is hydrophobicity of environments is high and protein charge is masked with the head of SDS monomer. The similar protective behavior of SDS was also reported in cytochrome C. At pH 4.0 urea were unable to unfold the cytochrome C when it was incubated with 2.0 mM SDS and its secondary as well as tertiary structures was not altered [37]. CD, fluorescence and DLS data revealed that if proteins are having charge on the surface, then urea interacts with protein directly and unfolding will occur on the another hand if the protein is in hydrophobic environment the urea is unable to interact directly with protein, but it will interact with environmental hydrophobicity. This lead us to conclude that urea

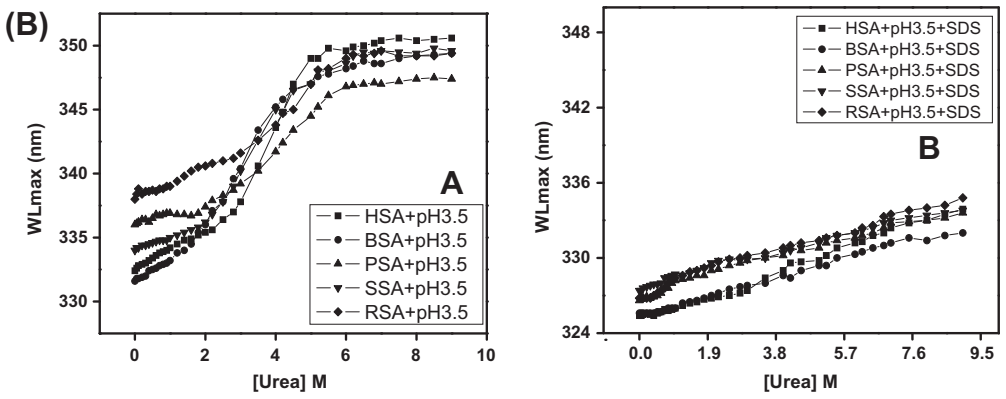

Fig. 2 (*continued*)

Table 1
Hydrodynamic radii of serum albumins were measured at different condition.

S. No.	Proteins	R_h (nm)						
		pH 7.4	pH 3.5	pH 3.5 + 4.0 mM SDS	pH3.5 + 5MU	pH3.5 + 5MU + 4.0 mM SDS	pH3.5 + 9MU	pH3.5 + 9MU + 4.0 mM SDS
1	HSA	3.4	4.0	3.2	5.1	3.7	9.3	4.3
2	BSA	3.7	4.5	3.1	6.2	3.9	11.3	4.8
3	PSA	3.9	4.4	3.9	5.2	4.5	14.3	5.0
4	SSA	3.4	4.4	3.9	5.8	3.9	12.4	4.5
5	RSA	3.4	4.0	3.6	6.0	4.1	10.8	4.6

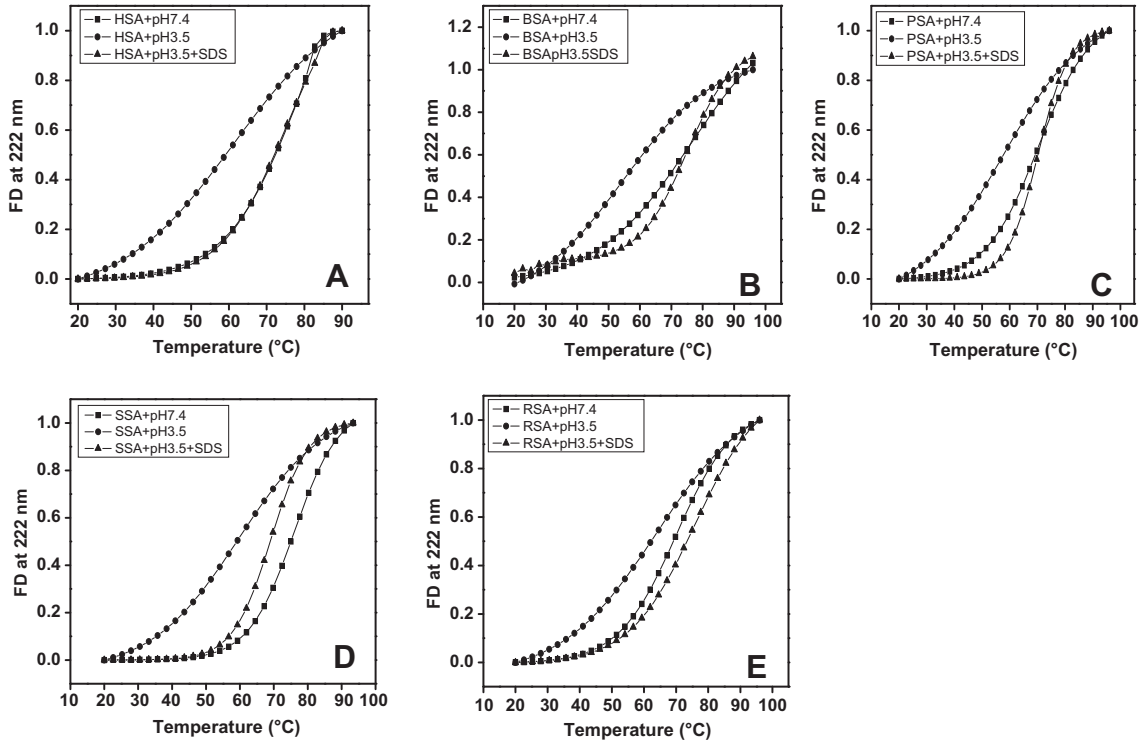

Fig. 3. Thermal denaturation of (A) human, (B) bovine, (C) porcine, (D) sheep and (E) rabbit serum albumins at pH 7.4 (-■-), pH 3.5 (-●-) and in the presence of 4.0 mM SDS at pH 3.5 (-▲-) measured by change in ellipticity at 222 nm. Albumin concentration was 5.0 μM. Before the measurements all the samples were incubated overnight.

interact with proteins directly if protein is having a charge on the surface while it interacts indirectly if the protein is in hydrophobic environments. Apart from urea denaturation, we also performed thermal denaturation for better understanding of the protective effect of SDS. Anionic surfactants are capable to protect the proteins against thermal denaturation [38,39]. The thermal denaturation of albumins was studied in the presence and absence of 4.0 mM SDS at pH 3.5 and same study was also performed at neutral pH. The albumins, whether at neutral or acidic pH, in the presence of 4.0 mM SDS showed sigmoidal transition with respect to increasing temperature. One thing is important to note that the albumins at pH 3.5 have very less thermal stability than at pH 7.4. While in

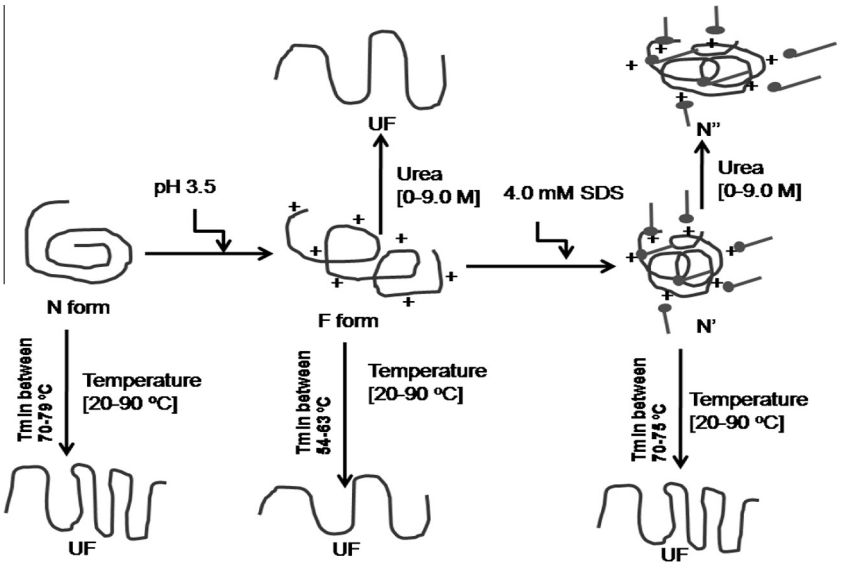

Fig. 4. Schematic presentation of SDS-stabilized albumins and their unfolding by urea and temperature.

case of SDS stabilized albumins, stability is similar to that of at pH 7.4 as evident from T_m values. Similar results were also found when BSA; was incubated with SDS the secondary structure of BSA was not disrupted even at 130 °C [38]. Earlier we have reported that RSA is very heat tolerable in the presence of 20.0 mM CTAB and RSA is not unfolded in the presence of CTAB [40]. From these observation we can concluded that the SDS is having strong protective effects against urea and thermal denaturation. Overall results are summarized in (Fig. 4) as graphical presentation.

Effect of SDS, urea and temperature in 'F' form of serum albumins was investigated. We found that 4.0 mM SDS concentration has great potential to protect the albumins from unfolding by urea (0–9.0 M). SDS is also showing a protective effect against temperature changes (20–90 °C). In the presence of 4.0 mM SDS, the urea was unable to unfold the serum albumins because SDS interacts with albumins via electrostatic as well hydrophobic interactions. From this study we have also proposed the mechanism of interaction of urea. Urea will interact with protein directly if the protein is in charged state otherwise indirect interaction will take place. Thermal stability is also provide by SDS due to electrostatic as well as hydrophobic interaction.

Stability of protein is a major concern in industries because some proteins are very unstable at physiological pH. This study will satisfy the stability problem.

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