Colloid Polym Sci 278:312–320 (2000)

ORIGINAL CONTRIBUTION

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Comparative investigations of the molecular properties of detergents and protein–detergent complexes

Received: 9 August 1999
Accepted: 19 October 1999

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Presented at the 39th general meeting of the Kolloid-Gesellschaft, Würzburg, 27–30 September 1999

Abstract Investigations of monomeric and micellar detergents, protein–detergent complexes, as well as native and denatured proteins by means of various physicochemical techniques yield a wide range of molecular characteristics of the components under analysis. Varying the experimental conditions (e.g., the concentration of solutes or the ionic strength of the medium) allows the mass, gross structure, and structural details of the macromolecular components to be determined. However, several modifications of the conventional techniques and evaluation procedures have to be applied in order to analyze multicomponent systems consisting of several low-molecular, micellar, and macromolecular components in an appropriate way. In the case of weakly absorbing detergents, labeling of the detergent micelles by specific dyes is required. Evidently, impurities and lack of homogeneity of many detergents may severely disturb the precise evaluation of the experiments; both necessitate a series of precautions in order to avoid misinterpretations. Analytical ultracentrifugation, size-exclusion chromatography, together with viscometry and densimetry, yield molar masses, mass distributions, and the overall structure of micellar and macromolecular molecules. In contrast, spectroscopic methods (UV absorption, fluorescence emission and excitation, far- and near-UV circular dichroism) monitor only local details of detergent-induced changes in the environment of aromatic residues. The technique of sodium dodecyl sulfate–polyacrylamide gel electrophoresis is routinely applied in biochemical work in order to establish molar masses of simple and conjugated proteins. To study the binding behavior of detergents to proteins in quantitative terms, however, techniques (e.g., equilibrium centrifugation, electrophoresis and chromatography) involving detergent concentrations have to be used.

Key words Ionic and nonionic detergents · Micelles · Protein–detergent complexes · Detergent binding · Physicochemical analysis

Introduction

Amphipathic molecules are ubiquitous in biological systems. Water, the most abundant solvent in living systems, proteins, membrane lipids, natural and synthetic surfactants, etc. represent molecules having both hydrophobic and hydrophilic moieties in chemical combination. The occurrence of amphipathicity at the molecular level gives rise to an extensive range of interactions in solution [1–8]. Many processes occur at interfaces, and the stability of functional systems, such as biological membranes, depends on the amphipathic nature of their constituents (membrane lipids, proteins, glycoproteins). Therefore, the investigation of these

types of amphipathic molecules, isolated or in combination, is a biologically significant objective.

The extensive use of nonionic and ionic detergents in preparative and analytical biochemistry demonstrates their importance in the biosciences. They serve as solubilizing agents for simple and conjugated proteins (glycoproteins). Electrophoresis in the presence of detergents, preferably sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), is frequently applied to analyze protein mixtures and to estimate the molar masses of polypeptide chains. Similarly, the physicochemical characterization, for example, by size-exclusion chromatography (SEC) or analytical ultracentrifugation (AUC), of protein–detergent complexes and their constituents is of considerable interest, both for a deeper understanding of the stabilizing forces and for the appropriate choice of detergents for a particular problem. The amphipathic nature of detergents is crucial for the process of micellization, for the occurrence of equilibria between free detergent monomers, detergent micelles, and protein–detergent molecules, and for their role as solubilizing/unfolding/denaturing agents.

Synthetic ionic detergents, such as SDS, tend to denature proteins, in contrast to natural ionic detergents and nonionic detergents. Since both ionic and nonionic detergents are amphipathic, the nature of the headgroup interactions must be responsible for the differences [1, 7]. The initial step of binding ionic detergents involves both Coulombic interactions with charged residues in the protein surface and hydrophobic interactions of alkyl chains with nonpolar amino acid residues. It is plausible that this initial binding occurs without significant changes in the tertiary structure of the protein. Increasing the detergent concentration, however, leads to enhanced binding and subsequent protein denaturation. The reason is that initially buried hydrophobic sites become exposed as preferred targets for further hydrophobic binding [1, 9, 10]. It is obvious that specific ionic binding is influenced by the pH or the ionic strength of the medium. In contrast, the binding of nonionic detergents is primarily hydrophobic; interactions of the alkyl chains being more important than those of the headgroups. This type of detergent–protein interaction is obviously too weak to denature proteins.

A wide variety of physicochemical techniques have been used for the characterization of detergent micelles and protein–detergent complexes: equilibrium dialysis, gel electrophoresis, SEC, spectroscopy [UV absorption, fluorescence, circular dichroism (CD)], hydrodynamics (AUC, viscometry, densimetry), solution scattering (light scattering, small-angle scattering), calorimetry, molecular dynamics, etc. – investigations aimed at determinations of mass, shape, and shape changes of the macrosolutes, and determination of the extent and nature of detergent binding to the proteins (binding isotherms). Though many techniques have been applied in the past, questions concerning the detailed structure of micelles and especially of protein–detergent complexes remain unanswered; these include, especially, the physical basis of detergent–detergent and protein–detergent interactions underlying the processes of self-organization under varying environmental conditions.

The present investigation is concerned with physicochemical investigations of various classes of monomeric and micellar detergents and of protein–SDS complexes as representative examples for protein–detergent complexes where drastic conformational changes take place upon detergent binding. For comparison, data on proteins denatured by strong chaotropic agents are included. For previous experiments in the same area see Refs. [11–19].

Materials and methods

Nonconjugated globular proteins, glycoproteins, and other reagents were of analytical grade. Poly(oxyethylene) alkylphenols (Nonidet P40, Triton X-100 and X-114), poly(oxyethylene) monoacyl sorbitans (Tween 20 and 80), and alkylthioglucoside (C_8TGS) were obtained from Boehringer (Mannheim), alkylglucosides (C_7GS–C_{10}GS) and dimethylalkylammoniopropane sulfonates (Zwittergent 3-08–3-16) from Calbiochem (Bad Soden), SDS from Serva (Heidelberg), and *N*-phenyl-1-naphthylamine (NPN) from Sigma (Munich).

Proteins and detergents were dissolved in bidistilled water or aqueous solutions containing 50–100 mM sodium phosphate buffer pH 7.0 (ionic strength about 0.1–0.2 mol/l). If necessary, for labeling (primarily micellar) detergents, the fluorescent dye NPN [20] was added. For SDS and protein estimates, the methylene blue [9] and Lowry [21] methods were applied. Investigations were performed at about 25 °C.

Absorption spectra were recorded using a Perkin-Elmer Lambda 5 spectrophotometer; difference spectra were created according to Ref. [22]. Fluorescence and CD spectra were recorded using a Perkin-Elmer MPF-44A spectrofluorometer and a JASCO J-500A spectropolarimeter, respectively. Spectral data were interpreted as described in Ref. [23].

SDS–PAGE was performed according to Laemmli [11], using slab gels. For SEC, a 120-ml Sephacryl S-300 high-resolution column (Pharmacia Biotech) was used. The conventional analysis by SDS–PAGE and SEC uses proteins preincubated with excess SDS and gels and buffers containing sufficient amounts of SDS to guarantee maximum binding. To investigate the interaction of SDS with proteins, methods working at variable concentrations of the detergent had to be developed [18]. Conclusions regarding binding isotherms require constant detergent concentrations in all parts of the system to maintain a stable thermodynamic equilibrium or the controlled alteration of the detergent concentration (detergent gradient). To interprete the binding behavior of the detergent, the amount of SDS was determined in all protein fractions of a SEC run in a separate experiment.

Sedimentation velocity and equilibrium experiments [24–26] were performed using a Beckman model E analytical ultracentrifuge equipped with a high-sensitivity photoelectric scanner, a multiplexer system, and a 10-in. recorder. Runs were performed in a six-hole rotor (AnG), using 12-mm double-sector cells (charcoal-filled epon) and sapphire windows. Velocity sedimentation is a hydrodynamic approach yielding information about mass and shape, whereas equilibrium sedimentation provides thermodynamic information with respect to mass distribution, aggregation, and intermolecular interactions. Sedimentation coefficients (s) of the

macrosolutes were obtained from the rate of movement of boundary indicator positions. Molar masses (M) and molar mass weight averages of the macromolecular species were determined by high-speed sedimentation equilibria (HSSE), using the meniscus-depletion technique [27]. To correlate the sedimentation coefficients of the globular particles with their molar masses, the proportionality between s and $M^{2/3}$ was used [28]. Partial specific volumes ($\bar{v}$) of the macrosolutes were taken from Refs. [13, 19, 29, 30]. Densities (ρ) and viscosities (η) of the solvents and the detergent solutions were determined using a Paar digital density meter (DMA 02) and an Ostwald viscometer, respectively. Intrinsic viscosities were calculated from approximations described in Ref. [31].

The investigation of micellar systems by means of AUC and SEC requires consideration of a number of precautions (summarized in Refs. [15, 16, 18, 19]). In connection with the present study, the following aspects have to be taken into account:

1. Detergents devoid of aromatic chromophores have to be labeled with appropriate (fluorescent) dyes in order to allow appropriate detection and analysis. The fluorescent dye NPN turned out to be a highly effective label in spectroscopic and ultracentrifuge studies, since it binds and/or is included into detergent micelles [14–16, 19]. For the investigation of protein–detergent complexes, however, these auxiliary means are not required because most proteins possess aromatic residues as intrinsic chromophores.
2. The investigation of protein–detergent complexes requires sufficient binding of the detergent to the proteins. On the other hand, concentrations of the free detergent above the critical micelle concentration (cmc) cause the formation of detergent micelles and disturb the analysis of protein–detergent complexes. This point was tested in a systematic manner in AUC and SEC experiments by varying the detergent concentration over a wide range so that the free detergent concentration was below or above the cmc.
3. A detailed analysis of SEC experiments with different equilibration buffers requires normalization of the elution volumes by means of a low-molecular compound.
4. The accuracy of mass estimates by HSSE is influenced by the correct choice of the baseline. Different approaches were tested, with the baseline position being varied between zero and the absorbance near the meniscus. The reference cells have to be devoid of macrosolutes (e.g., micellar detergents) to avoid skew baselines caused by different sedimentation in reference and solution cells.
5. The analysis of heterogenous systems may be achieved by experiments which cause particle separation. Both HSSE at different rotor speeds and SEC allow all the macrosolutes present in solution (detergent micelles, proteins, protein–detergent complexes) to be separated.

Results and discussion

Monomeric and micellar detergents

The absorption behavior of the various classes of detergents depends strongly on their chemical composition. Consequently, detergents containing aromatic chromophores (e.g., alkylphenols) exhibit pronounced absorption in the far-UV range between 250 and 300 nm, while detergents devoid of aromatic residues (e.g., various sulfates and sulfonates) exhibit only marginal absorption in this wavelength range (Fig. 1). Addition of a number of dyes, such as NPN, however, induces both a substantial enhancement of the overall absorbance of micellar detergents and pronounced spectral shifts. This helps the detection and analysis of the behavior of detergents devoid of aromatics.

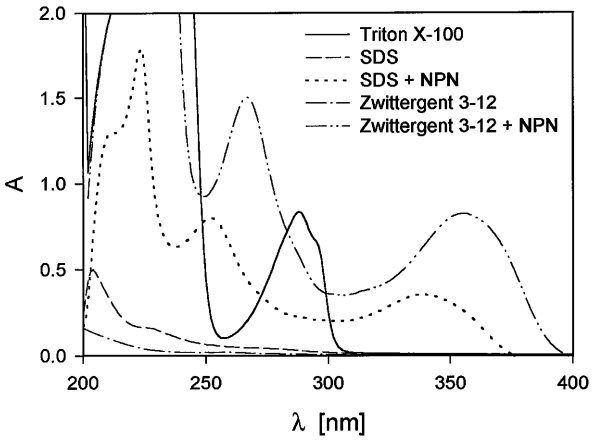

Fig. 1 Absorption spectra of aqueous solutions of selected nonionic and ionic micellar detergents in the absence and in the presence of 0.1 mM *N*-phenyl-l-naphthylamine (*NPN*). Triton X-100 (c = 0.033%) contains aromatic residues, whereas Zwittergent 3–12 (c = 1%) and sodium dodecyl sulfate (*SDS*) (c = 1%) are devoid of aromatics

The molecular properties of various classes of micellar detergents, such as partial specific volumes, $\bar{v}$, or sedimentation coefficients, s, are found to be correlated with the nature of the detergent class and the chain length [13, 19, 30], in addition to influences of size and shape of the micelles on s.

A large number of HSSE experiments yielded estimates of molar masses and aggregation numbers of various commercial detergents under a variety of experimental conditions (e.g., at different rotor speeds [19]). The results for various nonionic and ionic micellar detergents are summarized in Table 1. Though they are influenced by the accuracy of the values for $\bar{v}$ and ρ and by the correct baseline position, the accuracy of individual mass estimates is satisfactory ($\pm$ 5–10%). As they are taken from broad mass ranges in a number of cases, obviously, commercial detergent samples may exhibit pronounced heterogeneity. The application of different baselines, including detailed baseline iterations, shows that the observed mass distributions cannot be simulated by assuming homogeneity. A typical example is outlined in Fig. 2. The precise analysis of the molar mass distributions during or following the separation process in the centrifuge cells turns out to be an obvious advantage of AUC experiments. The values reported in the literature [19] are generally within the mass ranges given in Table 1; differences may be ascribed to differing experimental conditions and problems connected with the purity of the samples.

Table 1 Molar masses, M, and aggregation numbers, n, of selected nonionic and ionic micellar detergents in water or sodium phosphate (*NaP*) buffer obtained by high-speed sedimentation equilibrium (*HSSE*). Analytical ultracentrifugation (*AUC*) conditions: 10000–40000 rpm, scanning at λ between 230–295 nm and additionally at 346 nm in the presence of *N*-phenyl-l-naphthylamine (*NPN*), amplification factor (*AF*) = 1–4

Detergent				Solvent	AUC	
	M (g/mol)	c (%)	NPN (mM)		M_{mic}[a] (kg/mol)	n[b]
Nonionic detergents:						
Nonidet P40	606.8	0.05	–	H_2O	32–74	52–122
		0.05	–	NaP	33–84	53–139
Triton X-114	536.7	0.05	–	H_2O	91–104	170–193
Triton X-100	646.9	0.05	–	H_2O	52–57	80–88
		0.05	–	NaP	36–66	56–102
Tween 20	1227.5	1.0	–	H_2O	29–36	24–29
Tween 80	1309.7	1.0	–	H_2O	61–87	46–66
C_7GS[c]	278.3	1.0	0.1	H_2O	5–27	18–97
C_8GS[c]	292.4	0.5	0.1	H_2O	28–55	95–189
		0.5	0.1	NaP	26–84	88–287
C_9GS[c]	306.4	0.5	0.1	NaP	31–169	102–550
$C_{10}GS$[c]	320.4	0.5	0.1	NaP	38–58	119–180
C_8TGS[c]	308.4	1.0	–	H_2O	67	217
Ionic detergents:						
Sodium dodecyl sulfate	288.4	0.1	0.1	H_2O, 0.1 M NaCl	28–30	96–105
		0.1	0.1	NaP	23–26	80–90
		1.0	0.1	NaP	21–26	73–92
Zwittergent 3–08[c]	279.4	1.0	0.1	NaP	8–13	29–47
Zwittergent 3–10[c]	307.5	1.0	0.1	H_2O	20–33	64–108
		1.0	0.1	NaP	35–46	113–149
Zwittergent 3–12[c]	335.6	1.0	0.1	H_2O	30–65	88–193
		1.0	0.1	NaP	37–66	111–195
Zwittergent 3–14[c]	363.6	1.0	0.1	H_2O	47–108	129–298
		1.0	0.1	NaP	65–120	180–330
Zwittergent 3–16[c]	391.7	0.2	0.1	H_2O	73–158	187–404
		0.2	0.1	NaP	76–167	195–426

[a] Ranges of M_{mic} were obtained from sedimentation equilibrium experiments at varying experimental conditions (especially at different rotor speeds). They indicate a pronounced heterogeneity of the commercial detergent samples. In the case of sodium dodecyl sulfate relatively homogeneous samples were observed. For reference values and details on the accuracy of the data see Refs. [16, 19]
[b] Aggregation number calculated on the basis of the masses of the detergent monomers
[c] See text for an explanation of the names

Protein–detergent complexes and proteins in their native and denatured states

Experiments were performed with proteins (preferably albumins), either in their native state or under denaturing conditions, i.e., in the absence and presence of appropriate amounts of the anionic detergent SDS, or at high concentrations of typical chaotropic agents, such as guanidinium chloride (GdmCl), or at acidic pH.

The addition of SDS to albumins results in rather subtle changes in the UV absorption, characterized by a slight decrease in the maximum absorbance, a marginal blueshift (1–2 nm) of the maximum, and minute changes in the shape of the spectra (Fig. 3). The spectral peculiarities may be ascribed to partial denaturation caused by the action of the detergent. The hydrophobic aromatic chromophores of the protein core become solubilized by the detergent molecules and are then exposed to the polar solvent. This behavior may be visualized more clearly from the corresponding difference spectra, which show maxima at 276 and 284 nm. A comparison of the SDS-induced changes with experiments in GdmCl and at acidic pH reveal that the three transition curves are similar, however, the GdmCl effects are more pronounced than those caused by SDS or pH.

In contrast to the rather marginal changes in absorption upon addition of detergents, the changes in intrinsic fluorescence are very pronounced (Fig. 4): the fluorescence intensities are markedly decreased, showing substantial blueshifts of λ_{max} (as large as 30 nm). An inspection of several types of transition curves in different denaturants shows that GdmCl is a much more potent denaturant than SDS or pH. Obviously, addition of the detergent or acidic pH values yields only partial unfolding.

CD spectra in the far UV result in drastic changes in ellipticity upon addition of SDS (Fig. 5): in the case of bovine serum albumin (BSA) or human serum albumin (HSA), the θ amplitude is decreased, while in the case of ovalbumin (OvA) it is enhanced, corresponding to a

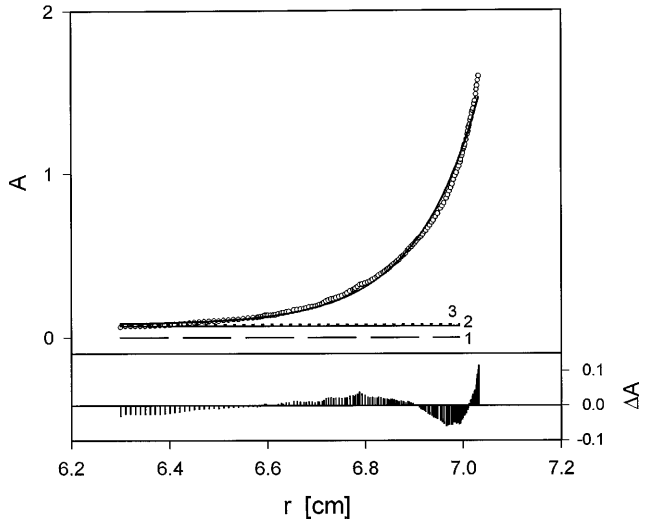

Fig. 2 High-speed sedimentation equilibrium of Zwittergent 3–12 micelles (c = 1.0%, H_2O, 0.1 mM NPN). *Upper panel*: experimental (○) and fitted (—) $A(r)$ data, together with different baseline positions. *1*: $A = 0$; *2*: $A = A$ (meniscus); *3*: A as a floating parameter to obtain the best linear fit in log A versus r^2 plots. Fitting assumptions: monodisperse system and exponential function of the type $a \cdot e^{br} + c$, where r is the radial distance and a, b and c are fitting parameters. *Lower panel*: local differences, $\Delta A(r)$, between experimental and fitted data. The recording of micelles was performed at 40000 rpm and 295 nm and an amplification factor of 1. Observed molar mass: 32.3 ± 2.5 kg/mol

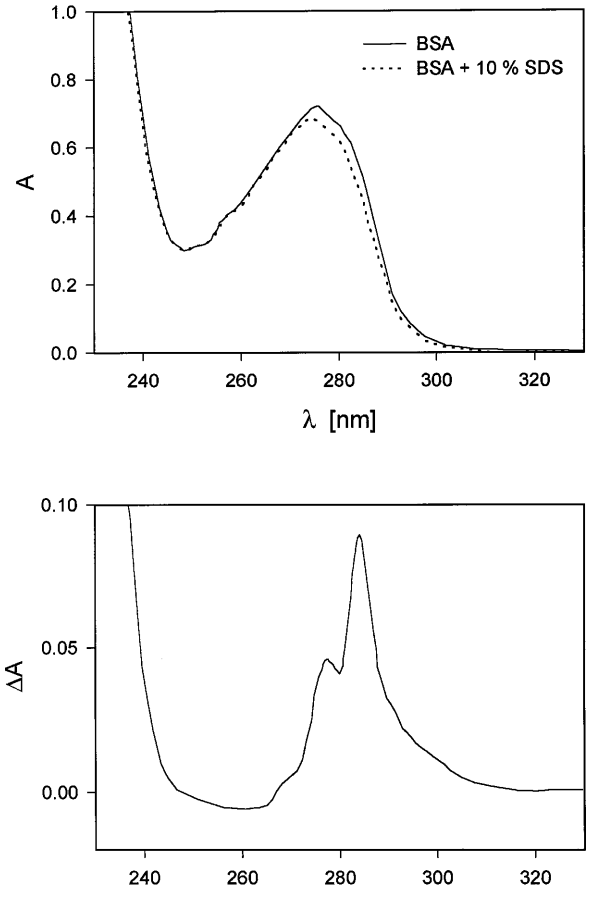

Fig. 3 UV absorption spectra of aqueous solutions of bovine serum albumin (BSA) [c = 1 g/l, sodium phosphate buffer (NaP)] in the absence and presence of 10% SDS. *Top*: spectra of native BSA and BSA–SDS complex. *Bottom*: difference spectrum BSA–SDS complex minus BSA + SDS

decrease in helicity for BSA and HSA and an increase for OvA. This observation is in agreement with the idea that proteins with different amounts of initial helix content may undergo "reconstructive denaturation" [32]. While upon SDS or acid denaturation the CD spectra only show changes in the secondary structure, GdmCl causes maximum randomization.

Near-UV CD spectra in the presence of SDS or at low pH show decreased signals for all three albumins, pointing to a decrease in tertiary structure (Fig. 6); again, GdmCl has a much stronger effect.

Conventional SDS–PAGE at varying acrylamide concentration (T) and evaluation in terms of Ferguson plots [17] may be used to unravel irregularities in the SDS binding behavior of proteins. The free electrophoretic mobilities (ordinate intercepts) are a measure of the amount of SDS bound to a protein. Most nonconjugated proteins show similar binding characteristics and share a common intercept. Obviously, the corresponding protein–SDS complexes have average properties, exhibiting uniform charge density and uniform overall conformation. In contrast, glycoproteins and other conjugated proteins exhibit abnormal (commonly lower) SDS binding compared to their nonglycosylated counterparts (Fig. 7).

At varying SDS concentration (but with constant detergent concentration in all parts of the electrophoresis system), PAGE of reduced and nonreduced proteins reveals differences in the migration behavior due to the difference in the extent of protein unfolding (Fig. 8). As a consequence, the resultant protein–SDS complexes exhibit different charge/volume ratios. Reduction, for example, by dithiothreitol (DTT), facilitates both unfolding and increased detergent binding.

Addition of SDS to BSA causes significant alterations of the intrinsic viscosity, [η], which is modulated by the ionic strength of the solvent and the presence of reducing agents, but not by further additives such as NaCl or saccharose (Fig. 9). Changes in [η] may be ascribed to detergent binding to the protein and detergent-induced unfolding. Different effects in buffer or water are due to different extents of hydration and electrostatic screening.

SEC experiments on protein–SDS complexes were performed using two different experimental approaches: application of native proteins (without any SDS incubation) to columns equilibrated with buffer containing different amounts of detergent (Fig. 10) and application

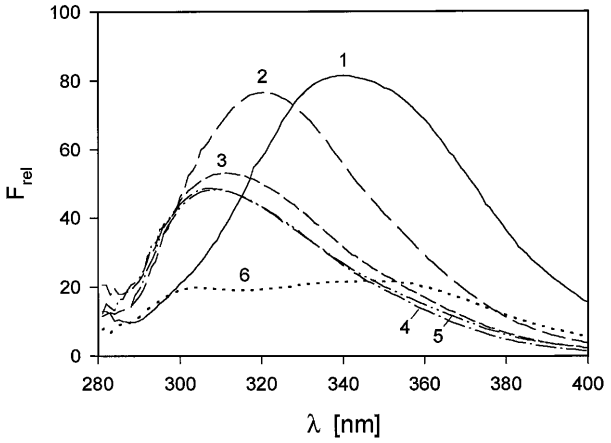

Fig. 4 Fluorescence emission spectra (λ_{exc} = 280 nm) of aqueous solutions of BSA (c = 1 g/l, NaP) in the absence (*1*) and presence of 0.01% (*2*), 0.1% (*3*), 1% (*4*), and 10% (*5*) SDS or 6 M guanidinium chloride (*GdmCl*) (*6*)

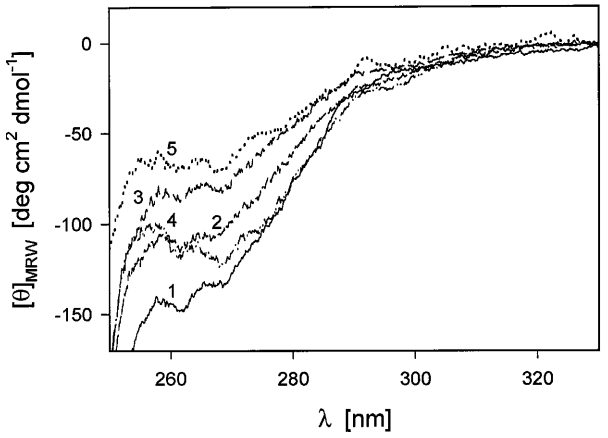

Fig. 6 Near-UV CD spectra of aqueous solutions of BSA (c = 1 g/l, NaP) in the absence (*1*) and presence of 10% SDS (*2*) or 10% SDS + 2 mM dithiothreitol (DTT) (*3*) or at pH 1 (*4*) or in 6 M GdmCl (*5*)

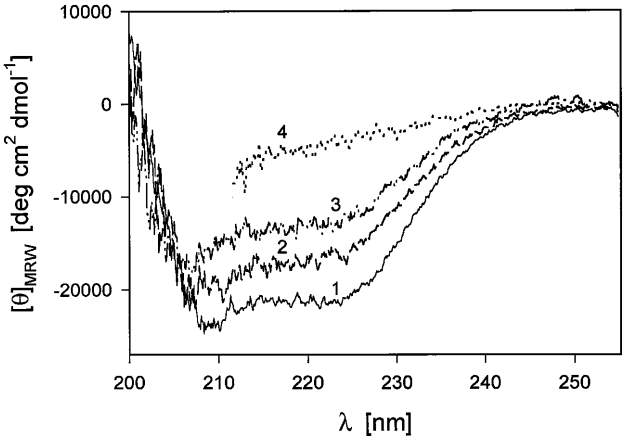

Fig. 5 Far-UV circular dichroism (*CD*) spectra of aqueous solutions of BSA (c = 0.1 g/l, NaP) in the absence (*1*) and presence of 10% SDS (*2*) or at pH 1 (*3*) or in 6 M GdmCl (*4*)

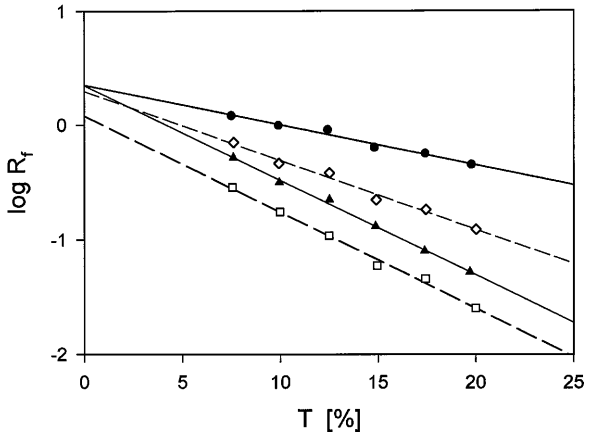

Fig. 7 Ferguson plot of selected nonconjugated and conjugated proteins obtained by evaluation of SDS–polyacrylamide gel electrophoresis (PAGE) at different acrylamide concentrations (T). Simple proteins: apoferritin (●), phosphorylase a (▲). Conjugated proteins: alkaline phosphatase (◇), α_2-macroglobulin (□)

of protein–SDS complexes (preincubated with SDS) to columns equilibrated with buffer of differing detergent concentrations. The first approach allows successive ligation of proteins, while the second approach is suited to quantify the amount of bound detergent. The binding ratios (grams of SDS/grams of protein) of selected proteins, obtained by the first approach, are shown in Fig. 11. The binding isotherms show a transition from a level of moderate binding (about 0.3–0.6 g/g) to a level of more pronounced binding (0.8–1.2 g/g for nonreduced proteins and about 1.2–1.8 g/g for reduced proteins). Obviously, the results depend strongly on the number of disulfides [33] and the reduction state of the proteins: with some reduced proteins, at elevated SDS concentrations, binding ratios up to 4 g/g are observed. The occurrence of two binding steps is in accord with previous findings and considerations of Reynolds and Tanford [9], Takagi et al. [34], and our own group [16, 18]. Our results indicate differences in the behavior of nonreduced and reduced proteins, suggesting a more gradual transition between the two binding levels than anticipated based on the binding isotherms reported by Tanford and Reynolds [2, 9]. Reducing agents, such as DTT, disrupt protein disulfides, thereby altering the tertiary structure of proteins and enhancing the extent of detergent binding substantially.

The sedimentation coefficients of protein–SDS complexes are considerably lower than those of their native counterparts. This behavior is attributable to partial unfolding and subunit dissociation (in the case of oligomeric proteins). For example, the BSA–SDS

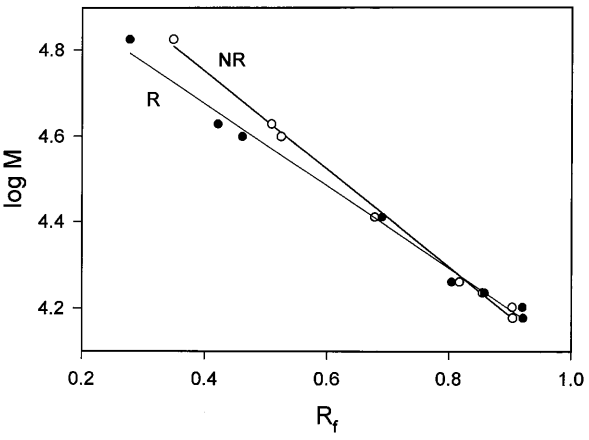

Fig. 8 Relative mobilities, R_f, of selected simple proteins obtained by PAGE at constant SDS concentration (0.015%). The data for nonreduced (*NR*) (○) and reduced (*R*) (●) proteins were approximated by regression lines

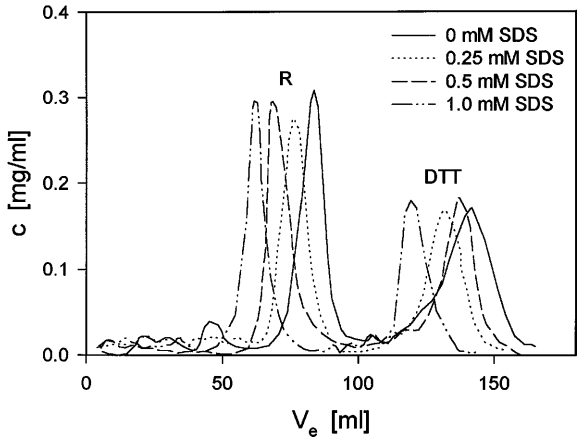

Fig. 10 Elution profiles of reduced ovalbumin (*OvA*), obtained by size-exclusion chromatography (*SEC*) runs with different SDS concentrations in the equilibration buffer

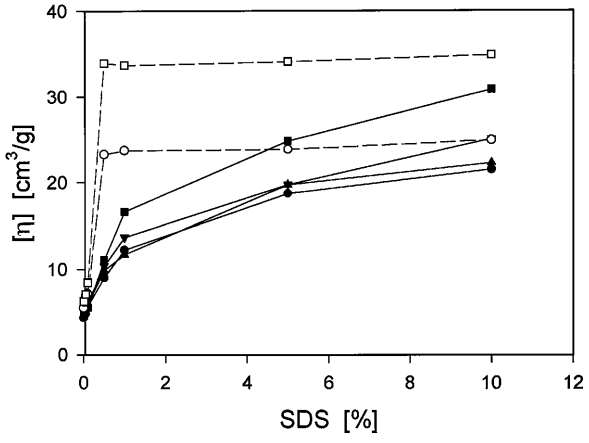

Fig. 9 Intrinsic viscosity, [η], of BSA–SDS complexes. Solvents and additives: NaP (●), NaP + 10 mM DTT (■), NaP + 100 mM NaCl (▲), NaP + 100 mM saccharose (▼), H_2O (○), H_2O + 10 mM DTT (□)

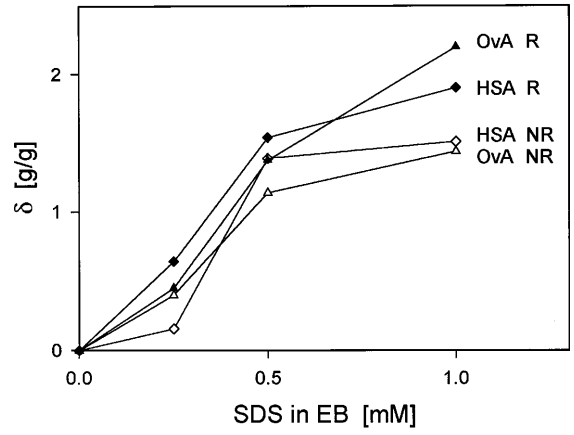

Fig. 11 Binding isotherms of selected simple nonreduced and reduced proteins at various SDS concentrations in the SEC equilibration buffer (*EB*). (*HSA*: human serum albumin)

complexes exhibit values of 4.1, 3.8, 3.6, and 2.8 S for 0.01, 0.1, 1, and 10% SDS, while a value of 4.5 S has been reported for native BSA [15, 35].

Sedimentation velocity runs of protein–SDS complexes may be used to obtain a rough estimate of the molar masses of native proteins [14, 15]. For this purpose, the following prerequisites are necessary: 1. Sedimentation coefficients of a series of marker proteins and of unknown proteins which have been solubilized by identical amounts of detergent. 2. The known molar masses of the marker proteins in their native state. 3. $s^{3/2}$ versus M plots. A typical example is shown in Fig. 12.

To study the behavior of protein–SDS complexes in more detail, HSSE experiments were performed on nonreduced BSA (1 g/l) by varying the SDS concentrations (0–1%) and the conditions of the sedimentation analysis (different rotor speeds and scanning conditions). Applying different rotor speeds under otherwise equal conditions allows the discrimination between particles of different size (Table 2). At the given protein concentration, SDS concentrations between 0.1 and 0.2% lead to free SDS concentrations below the cmc, still allowing sufficient binding of the detergent to the protein. The observed masses are caused by two forms of protein–SDS complexes, one corresponding to detergent binding of about 0.3–0.4 g/g and the other corresponding to about 0.8–0.9 g/g. Under the given conditions, obviously no further binding levels are observed, i.e., no definitive intermediate steps or higher levels of detergent binding are found in the course of the centrifugation experiments. Optimum conditions and minimum errors are achieved at SDS concentrations of about 0.09% SDS, where the mass determination of the

BSA–SDS complex is free from disturbances by detergent micelles at any rotor speed. At higher SDS concentrations the free detergent concentration is close to or above the cmc, thereby leading to the formation of detergent micelles which seriously perturb the estimation of the masses of the protein–SDS complexes. In this case, the resultant masses represent weight averages of both the micelles and the protein–SDS complexes; therefore, at high rotor speeds, anomalously low masses and large errors are observed. The HSSE results are in accordance with previous suggestions concerning SDS binding to proteins. For nonreduced BSA (containing 17 SS bonds) a maximum theoretical binding of 0.93 g/g is postulated [16, 33].

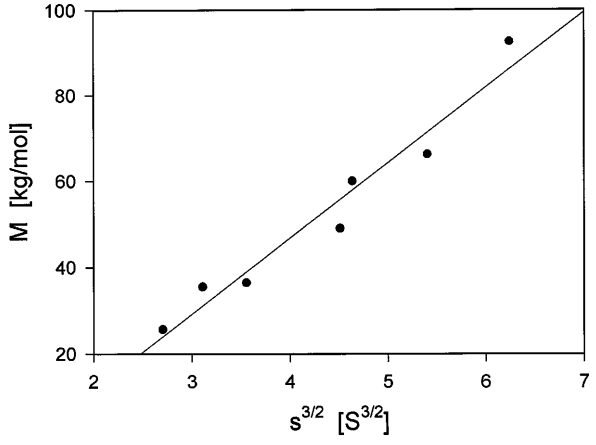

Fig. 12 Correlation between sedimentation coefficients of selected protein–SDS complexes (1% SDS) and the molar masses of the respective native proteins (devoid of detergent)

Sedimentation analyses of samples separated by SEC prior to the AUC experiment complemented the previously described SEC and AUC runs. Some eluates obained by SEC were subjected to HSSE runs in order to determine the molar masses of the respective protein–SDS complexes. Again, nonreduced and reduced proteins showed different properties but similar binding characteristics comparable to those observed in separate SEC or AUC experiments.

Conclusions

The analysis of micellar detergents and protein–detergent complexes by means of several physicochemical techniques discloses several molecular properties of these macromolecules. Depending on the chemical nature of the detergents and differences in the size and shape of their micelles, the results reveal different mass distributions, depending on the solvent conditions. Spectral and hydrodynamic characteristics are well suited to the analysis of local and global details, particularly in the case of protein–detergent complexes.

The investigation of protein–SDS complexes turns out to be extremely useful. While at low detergent concentrations the native structure of the protein component is affected only moderately, at elevated detergent concentrations extensive changes in the tertiary structure are observed. In the case of synthetic anionic detergents, such as SDS, electrostatic repulsion of the negative charges on the protein surface obviously leads to protein unfolding accompanied by changes to a variety of molecular parameters. Many SDS–induced effects are strongly influenced by the ionic strength of the medium,

Table 2 Molar masses, *M*, of native bovine serum albumin (*BSA*) (1 g/l, nonreduced) and BSA–sodium dodecyl sulfate complexes (NaP, absence of NPN) and amount of detergent binding, δ, to the protein obtained by HSSE under various experimental conditions. Detergent binding is based on the molar mass of native BSA of 66.3 kg/mol [35]. AUC conditions: 10000–40000 rpm, scanning at 230, 280, and 295 nm, AF = 1–4

Rotor speed (rpm × 10^{-3})	Sodium dodecyl sulfate										
	0%	0.09%		0.19%		0.37%		0.50%		1.00%	
	M (kg/mol)	*M* (kg/mol)	δ (g/g)	*M* (kg/mol)	δ (g/g)	*M* (kg/mol)	δ (g/g)	*M* (kg/mol)	δ (g/g)	*M* (kg/mol)	δ (g/g)
26	65 ± 2	83 ± 18	0.25	86 ± 25	0.30	68 ± 27[a]		44 ± 25[a]		56 ± 30[a]	
22	66 ± 3	88 ± 14	0.33	94 ± 44	0.42	75 ± 38[a]		78 ± 21[a]		73 ± 46[a]	
16	67 ± 0	87 ± 7	0.31	98 ± 24	0.48	88 ± 14[a]		103 ± 18[a]		86 ± 33[a]	
10	158 ± 59[b]	120 ± 7	0.81	126 ± 5	0.90	119 ± 7	0.79	127 ± 13	0.92	120 ± 11	0.81

[a] At free sodium dodecyl sulfate concentrations above the critical micelle concentration, the mass estimates at high rotor speeds are influenced by the presence of micelles ($M \approx$ 20–30 kg/mol, cf. Table 1); masses, therefore, represent weight averages of micelles and protein–sodium dodecyl sulfate complexes [16]

[b] Due to the unavoidable presence of cross-links (dimers and higher oligomers) in native BSA, at low rotor speed high molar mass weight averages can be found [16]; however, as a consequence of their enhanced size, the protein–sodium dodecyl sulfate complexes of the oligomers do not perturb the mass estimates of the complexes between monomers and detergent because the larger complexes are sedimented to the bottom at this speed

presumably involving changes in protein hydration [14, 36] and reduced detergent binding at low ionic strength [34]. Since SDS binding to proteins shows high affinity, further additives, such as NaCl or saccharose, have no significant influence on SDS-induced denaturation. In contrast, reducing agents, such as DTT, change the molecular properties of protein–detergent complexes significantly, if the native state of the proteins is stabilized by disulfide bonds. With respect to the denaturants tested, solubilization of proteins by SDS and acid denaturation leads to incomplete unfolding, whereas GdmCl shows a maximum chaotropic effect.

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