brief communication

# Protein structures in SDS micelle-protein complexes

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ABSTRACT Sodium dodecyl sulfate (SDS) is used more often than any other detergent as an excellent denaturing or "unfolding" detergent. However, formation of ordered structure (α-helix or β-sheet) in certain peptides is known to be induced by interaction with SDS micelles. The SDS-induced structures formed by these peptides are amphiphilic, having both a hydrophobic and a hydrophilic face. Previous work in this area has revealed that SDS induces helical folding in a wide variety of non-helical proteins. Here, we describe the interaction of several structurally unrelated proteins with SDS micelles and the correlation of these structures to helical amphiphilic regions present in the primary sequence. It is likely that the ability of native nonordered protein structures to form induced amphiphilic ordered structures is rather common.

## INTRODUCTION

The fact that proteins contain a large amount of secondary structure in the presence of SDS has been known for some time (Jirgensons, 1961, 1963, 1966, 1970, 1982). Additionally, the protein trichosanthin and the A-chain of ricin have been shown to demonstrate helical folding in the presence of SDS micelles (Kubota et al., 1987). More recently, SDS micelles were found to induce helical folding in apocytochrome *c* (Snel et al., 1991). Structural studies of these protein SDS complexes have revealed that the complexes are more helical than the native structure and that the overall particle shape consists of short rod-like segments connected by intervening flexible regions (Reynolds and Tanford, 1970; Tanford, 1980). Also, models have been proposed that describe in varying detail the structures present in the SDS micelle-protein complexes (Shirahama et al., 1974; Lundahl et al., 1986). However, structural analysis of the protein SDS micelles using CD has not been performed using an algorithm that can reasonably fit a spectrum to sheet, turn, and aperiodic content, as well as helix.

A number of studies (Lark et al., 1989; Pasta et al., 1990; Tsikaris et al., 1989; Wu and Yang, 1981) have demonstrated that a large number of amphiphilic peptides form helix in the presence of SDS micelles, whereas non-amphiphilic peptides do not.

It is, thus, of some interest to experimentally determine more accurately the extent of protein secondary structure in SDS micelle-protein complexes and the correspondence of this structure to amphiphilic regions of the protein. Here, we have calculated the amphiphilic potential of several structurally unrelated proteins using the helical hydrophobic moment developed by Eisenberg et al. (1982). The structure of each protein in the absence and presence of SDS micelles was calculated from circular dichroic (CD) spectra using the method of Manavalan and Johnson (1987). The results for porcine pepsin, yeast alcohol dehydrogenase, bovine carbonic anhydrase, bovine Immunoglobin G, human hemoglobin, and human serum albumin are presented.

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## MATERIALS AND METHODS

All spectra were taken in 10 mM sodium phosphate, pH 7.0, using a JASCO J600 spectropolarimeter (Easton, MD). Standard conditions were used for the measurements (Yang et al., 1986) with protein concentrations between 0.05 and 0.1 mg/ml. Concentration determination of protein solutions was by molar absorptivity at 280 nm (Fasman, 1976) except for immunoglobin, serum albumin, and hemoglobin, where molar ellipticity was used (Fasman, 1976). The method of Manavalan and Johnson (1987) was used to calculate the structural content of each protein from the CD spectra. Data from 184 to 240 nm at 1-nm intervals were used in the calculations.

Commercially available crystallized and lyophilized proteins were selected for study on a random basis. Proteins were excluded that were structurally similar to already selected molecules in order to avoid biased results. Salts and buffers present in the commercial protein preparations were removed by application of the protein solutions to a 1 × 27-cm Sephadex G-25 (Sigma Chemical Co., St. Louis, MO) column equilibrated with working buffer.

The helical amphiphilicity of each protein was calculated by the following equation according to Eisenberg et al. (1982):

$$\langle \mu_H \rangle = \left\{ \left[ \sum_{n=1}^{N} H_n \sin(\delta n) \right]^2 + \left[ \sum_{n=1}^{N} H_n \cos(\delta n) \right]^2 \right\}^{1/2} \Big/ N$$

where $\langle \mu_H \rangle$ is the helical hydrophobic moment, a quantitative measure of helical amphiphilicity, $n$ is a specific residue in a peptide segment of $N$ residues, $\delta$ is the distance between residues as viewed down the helical axis (100° for an α-helix), and $H_n$ is the hydrophobic value


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assigned to residue $n$. Normalized hydrophobic values (mean of 0 and standard deviation of 1) were assigned to each amino acid according to Eisenberg et al. (1984). The segment length ($N$) or window was defined as 11 amino acids and a "moving window" algorithm was used for all sequences as described by Rose (1978).

Amphiphilic regions were located according to the method of Parker and Song (1990), using the cutoff value of three or more consecutive residues having a hydrophobic moment greater than 0.35. This cutoff was assigned for the purpose of predicting helical regions in highly helical globular proteins, because regions with hydrophobic moments of this value or greater tended to form helical structure in the native molecule.

## RESULTS

In Fig. 1, *a* through *d*, there is a profound effect of SDS on the CD spectra of four non-helical proteins, in agreement with previous work (Jirgensons, 1982). All proteins exhibit the same basic spectral changes upon addition of SDS micelles. There is a large increase in negative ellipticity from ~200 to 250 nm. There is also a clear increase in positive ellipticity below 200 nm in all of these cases. Calculation of the secondary structural content of these four proteins reveals the same basic structural changes (Table 1). There is an average gain of ~10% β-turn structure and a loss of 2 to 10% coil structure. In the carbonic anhydrase, alcohol dehydrogenase, and pepsin analysis, there is a calculated increase of 9 to 16% in α-helix and a loss of ~15% β-sheet, roughly two-thirds of which is antiparallel. The immunoglobin structural change is approximately twice that of the other three non-helical proteins for both helix and sheet.

The CD spectra of hemoglobin (Fig. 1 *e*) and serum albumin (Fig. 1 *f*) with and without 40 mM SDS are indicative of an SDS-induced loss of α-helix, in agreement with previous results (Jirgensons, 1982). The results from the spectral analysis using the method of Manavalan and Johnson (1987) is shown in Table 1. Both proteins lost appreciable amounts of helix, but more than 90% of the helical content remains in both cases according to the CD analysis.

The amount of sequence ordered in a helical amphiphilic manner in the six proteins studied was determined as described in Materials and Methods (Table 1). Carbonic anhydrase, alcohol dehydrogenase, and immunoglobin all contain more amphiphilic helical regions (calculated) than are actually present in the protein as determined by the CD analysis (Table 1). Both pepsin and immunoglobin contain a number of regions not initially counted as amphiphilic that are calculated to be relatively high in helical amphiphilicity. These areas are not included in the initial value assignment of percentage amphiphilicity because they do not meet the cutoff value assigned by Parker and Song (1990) used for predicting helical regions in "all α-helical type" globular proteins. If these amphiphilic regions are included, the amount of calculated helical amphiphilic regions is increased to 33.9% of the total sequence in pepsin and 29.1% of the immunoglobin sequence (Table 1). Including these "moderately" amphiphilic regions, the percentage of calculated helical amphiphilicity corresponds more closely to the helical content (by CD analysis) of the SDS micelle-protein complex structure rather than the native protein structure for all four of the non-helical proteins analyzed (Fig. 1 *a* through *d*; Table 1).

The percentage of calculated amphiphilic helical regions in both human albumin and hemoglobin is somewhat less than the amount of helix indicated by the CD analysis. The reason for this is the binding of hydrophilic prosthetic groups on the normally hydrophobic interior of the molecule (Parker and Song, 1990). This interrupts the amphiphilic pattern (hydrophilic outside, hydrophobic inside). In contrast to the non-helical proteins, the percentage of calculated helical amphiphilicity in the two helical proteins is less than the percentage helix present in the native structures according to CD analysis (Table 1). Like the non-helical proteins, the percentage of calculated helical amphiphilic regions corresponds more closely to the helical content of the SDS micelle-protein complex than to the native structure.

## DISCUSSION

Using the hydrophobic moment (amphiphilicity) analysis of Eisenberg et al. (1982), it was reported that three out of seven proteins analyzed have a high potential to form amphiphilic α-helices even though no such structure exists in the native molecule (Parker and Song, 1990). The sequences involved are ordered in a statistically nonrandom fashion in order to form potential amphiphilic α-helices that are not present in the x-ray structure. The four proteins which did not possess this sequence characteristic were chromophore containing "all-α-helical" type proteins such as myoglobin and C-phycocyanin. In this study, we have found the same regions of amphiphilic sequence in four other non-helical proteins (carbonic anhydrase, alcohol dehydrogenase, immunoglobulin, and pepsin). These helical amphiphilic sequences are present in greater numbers than are helices in the native structure (Parker and Song, 1990; Table 1).

A number of authors have demonstrated that helical amphiphilic peptides form helix in the presence of SDS micelles, whereas non-amphiphilic ones do not (Lark et al., 1989; Pasta et al., 1990; Tsikaris et al., 1989; Wu and

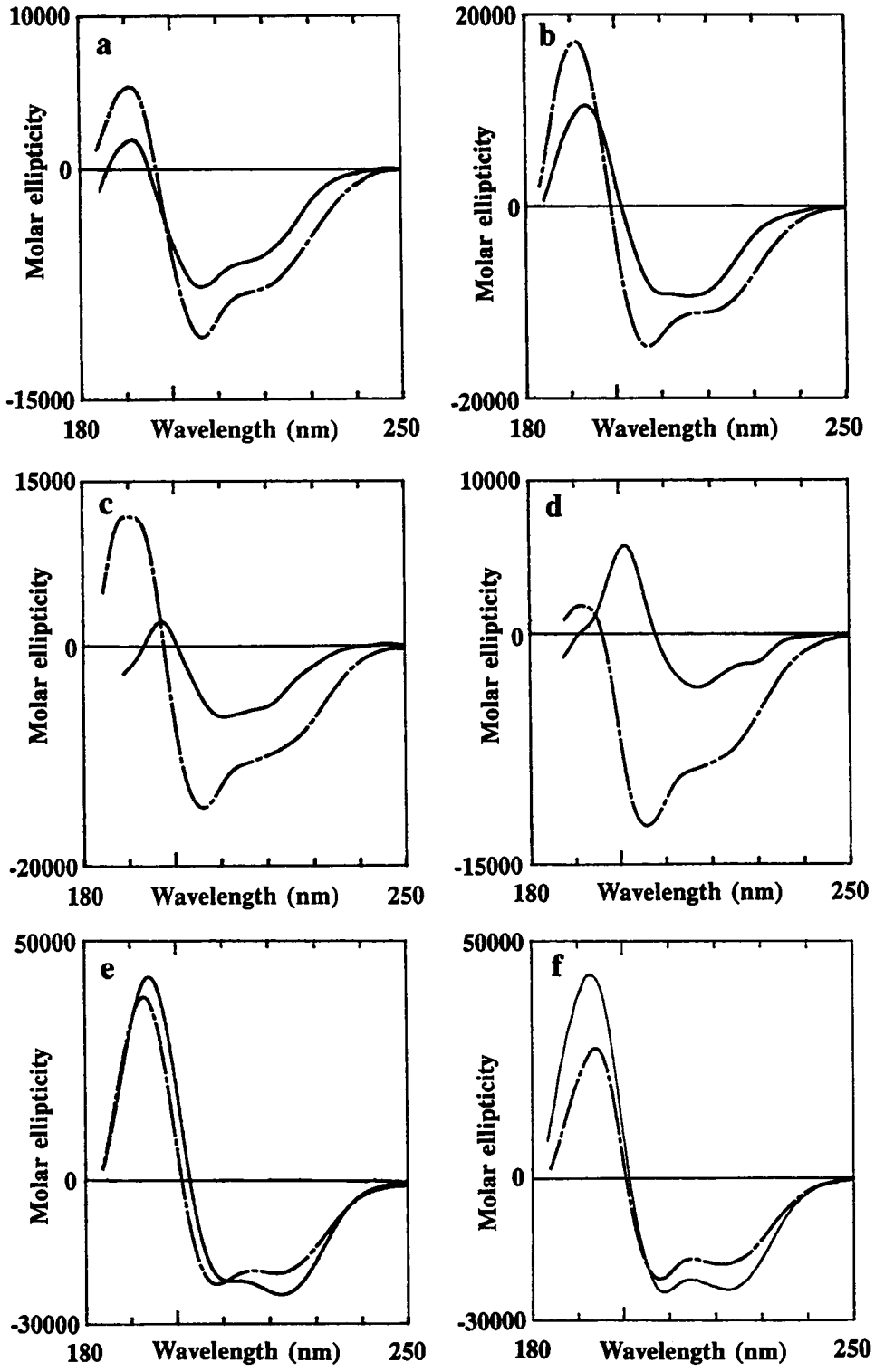


FIGURE 1 CD spectra with (----) and without (——) 40 mM SDS are as follows: (*a*) pepsin, (*b*) alcohol dehydrogenase, (*c*) carbonic anhydrase, (*d*) immunoglobulin G, (*e*) hemoglobin, and (*f*) albumin.

TABLE 1 **The percentage α-helix, antiparallel β-sheet, parallel, turns, and aperiodic (all other) structure calculated using the CD spectra in Fig. 1. The last column indicates the percentage of primary sequence ordered in an amphiphilic helical fashion according to calculation.**

| Protein | Helix | Antiparallel β-sheet | Parallel β-sheet | Turn | Other | Helical amphiphilic |
|---|---|---|---|---|---|---|
| Pepsin | 17 | 22 | 5 | 20 | 35 | 15.3 33.9* |
| Pepsin + SDS | 26 | 16 | 2 | 24 | 33 | |
| Alcohol Dehydrogenase | 28 | 19 | 6 | 15 | 32 | 34.9 |
| Alcohol Dehydrogenase + SDS | 37 | 10 | 1 | 25 | 26 | |
| Carbonic Anhydrase | 18 | 22 | 6 | 18 | 37 | 36.3 |
| Carbonic Anhydrase + SDS | 33 | 11 | 1 | 28 | 27 | |
| Immunoglobin | 6 | 29 | 10 | 12 | 42 | 11.6 29.1* |
| Immunoglobin + SDS | 31 | 10 | 0 | 26 | 36 | |
| Hemoglobin | 69 | 3 | −1 | 22 | 6 | 49.1 |
| Hemoglobin + SDS | 63 | 0 | 1 | 23 | 15 | |
| Albumin | 65 | −4 | −2 | 26 | 14 | 62.5 |
| Albumin + SDS | 62 | 4 | 0 | 18 | 13 | |

*These numbers include sequences that were calculated to be considerably amphiphilic although they did not meet the criteria described in Materials and Methods.

Yang, 1981). It may be assumed that, to some extent, proteins simply behave as "long peptides" in the presence of SDS because of the opening of the protein into a cylindrical structure (Tanford, 1980) that is closely associated with SDS. Thus, if helical amphiphilic regions (determined by calculation) are present in higher amounts than helix in the native structure, it is expected that there would be an increase in α-helix upon the addition of SDS. This is indeed the case for the four non-helical proteins studied (Fig. 1, *a–d*). As a negative control, two helical proteins which do not contain excess amphiphilic regions (determined by calculation) lost helix in the presence of SDS (Fig. 1, *e* and *f*; Table 1).

We did not test a large number of proteins and select only four that behaved in a similar fashion (Fig. 1). In addition to the proteins mentioned above, the only other protein tested was papain. Interestingly, under our conditions, this protein formed β-sheet (to be submitted for publication). It has previously been shown to form α-helix in the presence of SDS under different buffer conditions (Jirgensons, 1963). Papain contains a large amount of both helical amphiphilic and β-strand amphiphilic sequences that do not form helix or strand in the native structure.

In summary, proteins which contained a markedly high amount of primary sequence ordered with respect to helical amphiphilicity underwent the same basic structural changes upon addition of SDS. This change includes an increase in α-helix and turn, as well as a loss of β-sheet and unordered structure.

An important question remains: do these aperiodic (native structure) sequences with ordered amphiphilic potential have any biological function? It has been proposed that these sequences may be involved in transient structures present only during folding (Parker and Song, 1990). Another very intriguing possibility is that these sequences are important for interaction with chaperonins. Chaperonins have been labeled as "protein detergents" and are widespread and abundant in biological systems. As a chaperonin interacts with a denatured molecule that has been caught in a dead end folding path or potential energy well, various amphiphilic sequences may be docked (hydrophobic collapse) onto a chaperonin in a structurally ordered and thus stable manner. This docking might involve the same nonnative but ordered secondary structures that are involved in SDS micelle-protein interactions.

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