J. Biosci., Vol. 4, Number 3, September 1982, pp. 331-345. © Printed in India

Studies on the conformation of α-globulin from *Sesamum indicum* L. in cationic and nonionic detergents*

V. PRAKASH and P. K. NANDI†

Protein Technology Discipline, Central Food Technological Research Institute, Mysore 570 013

†National Institute of Arthritis Metabolism and Digestive Diseases, National Institute of Health, Bethesda, Maryland 20014, USA.

MS received 19 November 1981; revised 19 May 1982.

Abstract. The circular dichroic spectra of α-globulin from *Sesamum indicum* L. was recorded in the presence of cetyltrimethyl ammonium bromide, Triton X-100 and Brij-36T. The protein in 0.2 M phosphate buffer pH 7.4 had about 25% β-structure and 5% α-helix, the rest being aperiodic or irregular structure and α-helix, structure was increased by cationic detergent cetyl trimethyl ammonium bromide. But, the increase in α-helix content was much less than that induced by an anionic detergent, sodium dodecyl sulphate. In non-ionic detergent like Brij-36T and Triton X-100, specific β-structures like II-β and I-β were formed along with changes in α-helical and aperiodic structures. These results suggest that the protein has a fairly labile quaternary structure.

Keywords. Circular dichroism; α-globulin; cationic detergent; nonionic detergent; dissociation and denaturation; I-β and II-β structures.

Introduction

The major protein of *Sesamum indicum* L. α-globulin, constitutes ~ 65-70% of the total proteins present in the seed. The molecular weight, the association-dissociation and denaturation behaviour in the presence of various solution conditions of electrolytes, detergents, urea, guanidine hydrochloride, acid and alkali of the isolated fraction was characterized (Nath *et al.,* 1957; Sinha and Sen, 1962; Ventura and Lima, 1963; Prakash, 1976; Prakash and Nandi, 1976 a,b; 1977 a,b,c; 1978; Prakash *et al.,* 1980; Prakash, 1980; Lakshmi and Nandi, 1977,1978). Recently it was shown that the multimeric α-globulin had a labile secondary structure with a fair degree of conformational freedom and had nearly 70% aperiodic structure, 25% β-structure and about 5% α-helical structure (Prakash, 1980; Prakash *et al.,* 1980). Reagents such as acid, alkali, urea and guanidine

*Part of this work was presented at the Second FAOB Congress and Golden Jubilee Meeting of the Society of Biological Chemists (India) held during December 14-18, 1980 at Bangalore, India (*Indian J. Biochem. Biophys.*) and the work was done at the Biochemistry Department, Brandeis University, Waltham, Massachusetts 02254 USA.

Abbreviations used: SDS, Sodium dodecylsulphate; CD, circular dichroism; CTAB, cetyltrimethyl ammonium bromide.

hydrochloride, caused a significant change in the conformation of α-globulin to more of aperiodic structure as determined by circular dichroism (Prakash *et al.,* 1980). On the other hand, in anionic detergents like sodium dodecyl sulphate (SDS), the protein attained more of ordered structure even at 10 mM SDS (Prakash *et al.,* 1980).

Cationic detergents are used in solubilising membrane proteins (Heller, 1968; Bornet and Edelhoch, 1971; Williams and Gratzer, 1971; Nakaya, *et al.,* 1971; Hong and Hubbell, 1972; Birdi, 1973; Nozaki, *et al.,* 1974; Aoki and Hiramatsu, 1974; and Ushiwata *et al.,* 1975). Also the nature of interaction between nonionic detergents and proteins has received considerable attention in recent years as these reagents also solubilise membrane proteins without disrupting the native structure of the proteins isolated or causing loss of their biological activity (Helenius and Simons, 1971; 1972; Makino and Tanford, 1973; Makino *et al.,* 1973; Helenius and Simons, 1975; Dunnkbey *et al.,* 1976; Shukolynkov *et al.,* 1976; Robinson and Capaldi, 1977; Jirgensons, 1980).

In the presence of cationic detergent cetyl trimethyl ammonium bromide (CTAB), α-globulin was shown to undergo aggregation followed by dissociation and ultimately denaturation (Lakshmi and Nandi, 1977). Also the pK_{int} of phenolic groups of the protein in the presence of 10 mM CTAB suggested that a conformational change in the protein molecule might have taken place (Lakshmi and Nandi, 1977). On the other hand, in the presence of nonionic detergents Triton X-100 and polyoxyethylene 10-lauryl ether (Brij-36T), dissociation and aggregation of α-globulin occur (Lakshmi and Nandi, 1978). However, no change in the pK_{Int} of phenolic groups of the proteins or the viscosity of the protein was observed even at 1 mM Triton X-100 (Lakshmi and Nandi, 1978) indicating a characteristic difference between the two classes of detergents either in stabilizing or destabilizing the native conformation of α-globulin.

The present study describes work aimed at investigating the conformational stability of α-globulin in solution in the presence of CTAB, Triton X-100 and Brij-36T. Ultimately, this information would help a better understanding of the inter- and intra-molecular forces which hold the various subunits of α-globulin as a thermodynamically stable protein unit.

Materials and methods

Sesame seed of the variety *Sesamum indicum* L. was purchased locally. CTAB, Triton X-100 and Brij-36T were from Sigma Chemical Co., St. Louis, Missouri, USA. All the reagents were of Reagent grade.

Isolation of α*-globulin*

α-Globulin was isolated by the procedure described earlier (Prakash and Nandi, 1978).

Protein concentration

Protein concentration was determined by using a value of 10.8for $\varepsilon_{1\,CM}^{1\%}$ at 280 nm (Prakash and Nandi, 1978).

Circular dichroism

Circular dichroic (CD) spectra were recorded with a Gary 60 spectropolarimeter with a model 6001 attachment. Slits were programmed so as to yield a 1-nm band width at each wavelength. The data were reduced to mean residue ellipticites (Θ), using a digital PDP-11 computer and using a value of 115.5 for the mean residue weight for α-globulin calculated according to the amino acid analysis reported elsewhere (Prakash and Nandi, 1978). In the presence of Triton X-100, CD spectra could not be obtained in the near ultraviolet region (360-250 nm) due to the interference of Triton X-100 in the above region as a result of which the noise level was fairly high. The near ultraviolet CD curves in the presence of CTAB and Brij-36T were resolved into gaussian bands by the Du Pont Model 310 curve resolver. The far ultraviolet CD curves were curve fitted using the Digital PDP-11 computer by Greenfield-Fasman procedure (Greenfield and Fasman, 1969). The best-fit curve was taken as the one which had least error and which included most of the experimental points of the far ultraviolet CD curve. A protein concentration of 12.5 μM at various concentrations of CTAB and 5.8 μM at various concentrations of Brij-36T were used for the near ultraviolet CD spectra, using a 1 cm pathlength cell. For the far ultraviolet CD spectra, protein concentration of 12.5,6.7 and 5.8 μM in the presence of CTAB, Triton X-100 and Brij-36T respectively was used with a 0.01 cm pathlength cell. The spectra were recorded at 25±1°C. The dichroic absorbance differences were averaged from two to three recordings and the mean residue ellipticity was calculated from the averaged spectrum.

All the spectra were obtained in 0.2 M phosphate buffer, pH 7.4. The concentrations of the detergents mentioned is the free concentration of the respective detergents.

Results

The near ultraviolet CD spectrum of α-globulin from 330-250 nm in 0.2 M sodium phosphate buffer, pH 7.4 is shown in figure 1. The spectrum was characterized by a major positive peak at 284 nm with shoulders at 290, 265 and 256-258 nm and a negative peak at 204 nm.

The far ultraviolet CD spectrum of α-globulin from 255 to 200 nm is also shown in figure 1. The spectrum contained a major negative peak around 210 nm. The absence of any fine structure in the spectrum probably indicated the dominance of aperiodic and β-pleated structure in the secondary structure of α-globulin. This is in excellent agreement with the results published earlier (Prakash *et al.,* 1980) and the assignments of the various bands was made as described earlier (Prakash *et al.,* 1980).

In the above procedure, the data is curve fitted by Greenfield-Fasman Procedure (1969) by comparison of the far ultraviolet CD spectra of poly-L-Lysine-Histone standards and/or Yang's standards as the reference spectra. Based on this, the percentage of α-helix, β-and aperiodic structure is calculated for the protein. These values are utilized at best to indicate only the order of magnitude in testing conformational transitions upon variation of solvent systems.

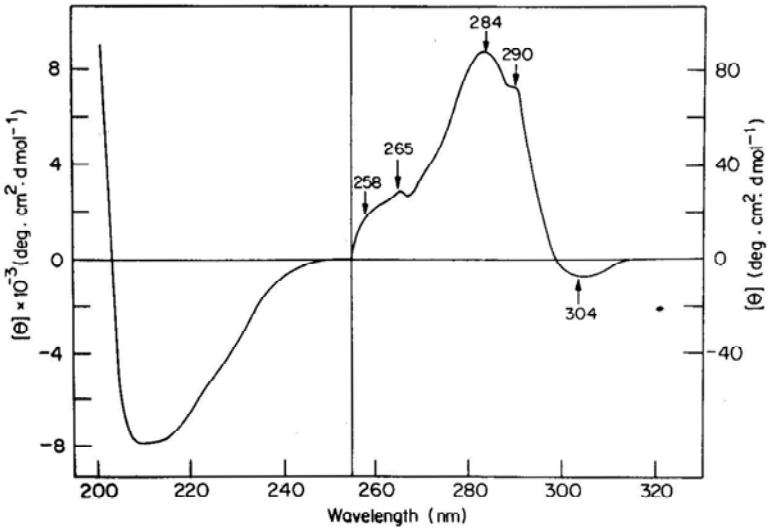

Figure 1. Circular dichroism spectra of α-globulin in 0.2 M sodium phosphate buffer, pH 7.4, in the 190-330 nm region.

An analysis of the far ultraviolet CD spectra of α-globulin in sodium phosphate buffer 0.2 M, pH 7.4 by the procedure of curve fitting indicated that α-globulin consisted of nearly 5% α-helix, 25% β-structure and the remaining aperiodic structure, indicating a similarity in α-globulin structure with the other major storage seed proteins (Jirgensons, 1963a; Jacks *et al.,* 1973; Koshiyama, 1972; and Prakash *et al.,* 1980; Prakash and Narasinga Rao, 1982).

Effect of cationic detergents

The aggregation, dissociation and denaturation of α-globulin in the presence of CTAB has been investigated in detail from our laboratory (Lakshmi and Nandi, 1977). The results indicated that upto a mole ratio of 100 mol of [CTAB] total per mol protein, the protein precipitated out of solution above which redissolution of the protein occurred (Lakshmi and Nandi, 1977). Velocity sedimentation results indicated the presence of higher aggregates in the system at CTAB concentrations of 0.05-1.0 mM. Also the various spectral measurements indicated that the chromophores on the protein were perturbed probably due to conformational change in the protein. In order to look into such conformational changes in the protein, the CD spectrum of the protein was obtained at 0.1,1.0 and 10 mM CTAB.

Figure 2 shows the near ultraviolet CD spectrum of α-globulin in 0.1 mM CTAB in 0.2 M sodium phosphate buffer, pH 7.4. The spectrum was characterized by a nearly 12% decrease in the intensity of the major peak as compared to that of the protein in phosphate buffer alone. New CD peaks are present at 289,282,276 and 270 nm. Also, the fairly sharp negative transition at 304 nm in the control protein is transformed into a broad negative peak spreading from 320-300 nm in the presence of 0.01 mM CTAB. The above results indicate that the tryptophan and

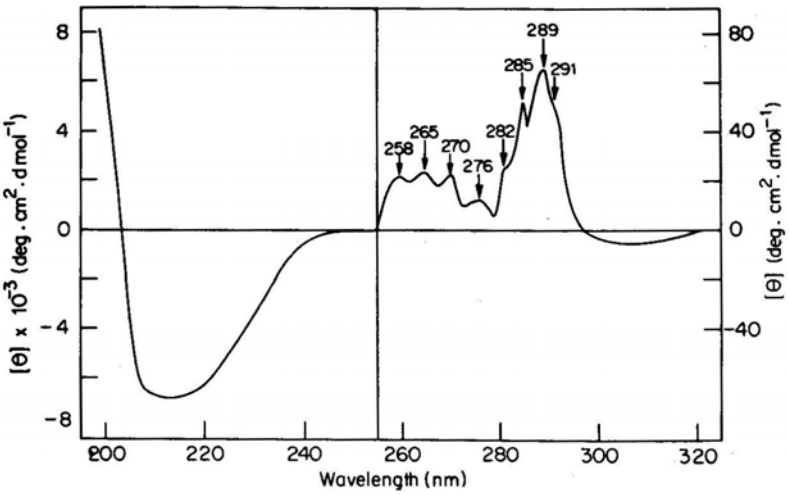

Figure 2. Circular dichroism spectra of α-globulin in 0.1 mM CTAB in 0.2 M sodium phosphate buffer, pH 7.4 in the 190-325 nm region.

tyrosine residues are significantly perturbed at this concentration of CTAB. At this concentration of CTAB, nearly 50% of α-globulin is present as 13 S component in 0.1 M Tris-HCl buffer, pH 8.6, the remaining protein being the dissociated 8 S component and the aggregated 30 S and ~230 S components as observed in velocity sedimentation experiment (Lakshmi and Nandi, 1977). The origin of the above CD spectral change could be due to the association-dissociation of the protein where the tyrosine and tryptophan residue are intimately involved. Further, the significant change in the 304 nm band can be attributed to a more asymmetric environment around the tryptophan residues probably arising out of the binding of charged detergent molecules as well as the change in the microenvironment arising from preferential hydration or change in the dielectric constant of the medium (Strickland, 1974; Prakash *et al.,* 1980). Further, the new peaks in the 250-270 nm region could also arise from the dihedral angle of disulphide, the C-S-S bond angle and the vicinal interactions (Lindberg and Michl, 1970; Timasheff, 1970a, b; Webb *et al.,* 1973; Sears and Beychok, 1973; and Casey and Martin, 1972).

The far ultraviolet CD spectrum of α-globulin under the above mentioned conditions, is also shown in figure 2. A comparison of the far ultraviolet CD spectrum of the protein in sodium phosphate buffer and in 0.1 mM CTAB indicates a decrease in the α-helical content of the protein as also of the aperiodic structure. The curve fitting of the data indicates that the protein has slightly increased α-helical content but the increase is not much as compared to the anionic detergent SDS which induces nearly 20% α-helix at 1.0 mM SDS (Prakash *et al.,* 1980). This has been observed with other proteins also (Jirgensons, 1967; Verpoorte and Kay, 1966; Lederer, 1968; Ikeda and Hamaguchi, 1970; Timasheff, 1970a, b; and Viser and Blout, 1971). The percentage of α-helix, β- and aperiodic structure is given in table 1. No decrease in aperiodic structure is indicated in the curve fitting procedure, this may also be due to the compensatory effect of the β-structure.

Table 1. Secondary structure of α-globulin at 24-26°C under various solvent conditions[a]

No.	Solvent	Structure (%)		
		α-Helix	β	Aperiodic
1.	Sodium phosphate buffer, pH 7.4, 0.2 M	5	25	70
2.	CTAB (mM)			
	0.1	10	16	74
	1.0	11	13	76
	10	5	10	85
3.	Brij-36T (mM)			
	0.1	15	15	70
	1.0	10	9	81
	10	10	21	69
4.	Triton X-100 (mM)			
	0.01	10	12	78
	1.0	10	12	78
	10	13	8	79

[a] Estimated by curve fitting to far ultraviolet CD spectra using Greenfield-Fasman procedure (1969).

The near ultraviolet CD spectrum of α-globulin at 1.0 mM CTAB is shown in figure 3. From the figure it is apparent that there was a further decrease in the amplitude of the overall CD spectrum. The above results in conjunction with the results at 0.1 mM CTAB concentration indicated a further change in the spectral properties of the chromophores at this concentration of CTAB.

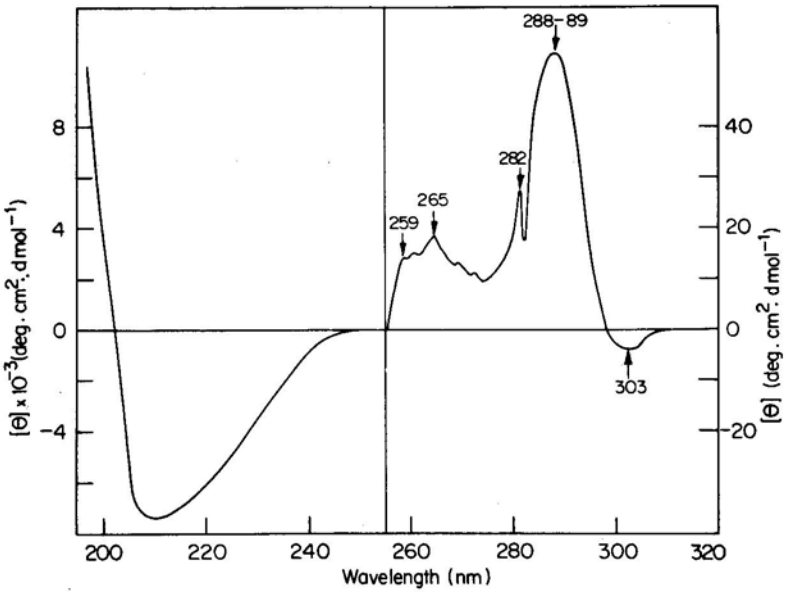

Figure 3. Circular dichroism spectra of α-globulin in 1 mM CTAB in 0.2 M sodium phosphate buffer, pH 7.4 in the 190-320 nm region.

The far ultraviolet circular dichroic spectrum is also shown in figure 3. The spectrum looks every similar to that at 0.1 mM CTAB except that the 210 nm band was shifted to 209 nm and was less broad. The curve fitting of the data indicates 76% of aperiodic structure, 13% β-structure and 11% α-helix structure.

The near ultraviolet CD spectrum of α-globulin at 10 mM CTAB concentration is shown in figure 4. The amplitude of the peaks remains the same as at 1.0 mM CTAB. However, there was a much better resolution of the 290,287,275,266 and 258 nm peaks. Also the negative peak at 304 nm was blue-shifted to 302 nm. At a

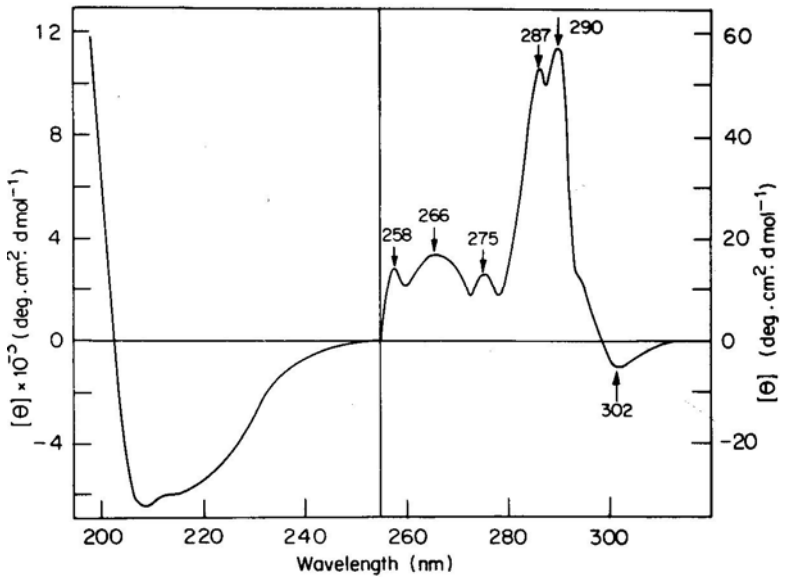

Figure 4. Circular dichroism spectra of α-globulin in 10 mM CTAB in 0.2 M sodium phosphate buffer, pH 7.4 in the 190-320 nm region.

similar concentration of SDS, the protein was devoid of any CD bands in the near ultraviolet region indicating a gross difference in the conformation of α-globulin in the presence of anionic and cationic detergents (Prakash *et al.,* 1980).

Figure 4 also shows the far ulraviolet CD spectrum of α-globulin in presence of 10 mM CTAB. A small change in the amplitude at210 nm is observed and210 nm original CD is blue-shifted to 208 nm band indicating increased aperiodic structure in the protein. The curve fitting of the data showed that nearly 85% of the protein was of aperiodic structure, 12% β-structure and 5% α-helical structure. A comparison of the spectra indicated a decrease in the α-helix structure of the protein though not significant, and could possibly be envisaged in various kinds of aggregates that are found at this concentration of CTAB. However, the predominant structure at this concentration of CTAB was aperiodic as compared to SDS where at a similar concentration, the protein has nearly 20% α- helix, 10% β-structure, the remaining being aperiodic (Prakash *et al.,* 1980).

Effect of non-ionic detergents

The nonionic detergents Triton X-100, Brij 36T (polyoxyethylene 10 lauryl ether) was shown to dissociate and aggregate α-globulin above critical miscellar concentration of the detergent (Lakshmi and Nandi, 1978). Spectroscopic measurements indicated no change in the environment of the tryptophan and tyrosine inspite of dissociation and aggregation of the protein in the presence of either Triton X-100 or Brij-36T (Lakshmi and Nandi, 1978). Binding measurements suggested that perhaps micelles of the detergent bind predominantly to the exposed hydrophobic surfaces of the protein subunits. However, viscosity measurements showed no major conformational change of the protein in the detergent solutions (Lakshmi and Nandi, 1978). Hence it was of interest to look into the near and far ultraviolet CD spectra of α-globulin in the presence of the above nonionic detergents to obtain an insight into the conformational stability of the protein in the presence of these detergents. This was of special interest, since as mentioned earlier, these detergents are known neither to disrupt the native structure nor cause any loss in their biological activity (Thompson and Bachelard, 1970; Alexander and Penefsky, 1971).

(A) Brij-36T

The near ultraviolet CD spectrum of α-globulin in 0.01 M Brij-36T is shown in figure 5. The CD spectrum was very similar to that of the CD spectrum of α-globulin in sodium phosphate buffer alone except that the negative trough at 304 nm was broader. This observation is in excellent agreement with the spectrophotometric and fluorescence measurements where no change has been

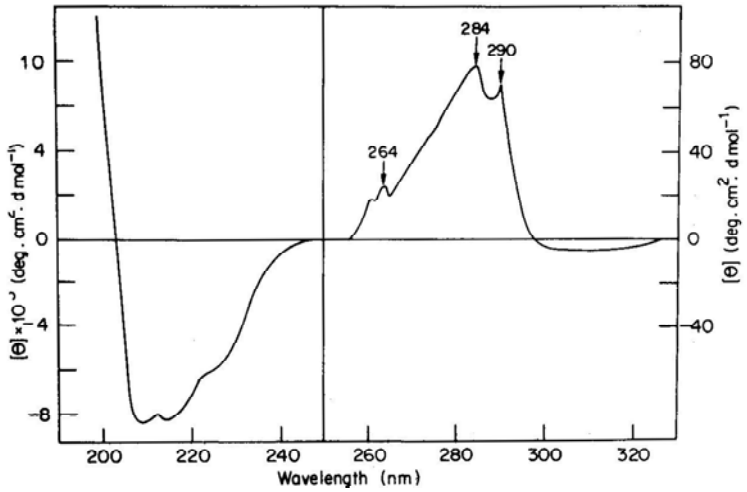

Figure 5. Circular dichroism spectra of α-globulin in 0.1 mM Brij-36T in 0.2 M sodium phosphate buffer, pH 7.4 in the 190-325 nm region.

observed in the environment of tyrosine and tryptophan (Lakshmi and Nandi, 1978). However, the small change of the asymmetric environment around the

tryptophan residues due to the binding of detergent molecules producing a change in the preferential hydration might be responsible for the alteration in the CD trough at 304 nm.

Figure 5 also shows the far ultraviolet CD spectrum of α-globulin in the presence of 0.01 M Brij-36T. The peaks had a larger intensity as compared to that of the protein in buffer alone. Also, the fine structure of the spectra was enhanced with a CD band appearing as a shoulder at 226 nm and well resolved troughs at 209 nm and 215 nm indicating the generation of a more ordered structure in the protein. The curve fitting analysis showed 15 % α-helix, 15 % β-structure and the balance being aperiodic structure (table 1). Although a nonionic detergent like Brij-36T is known not to disrupt the native structure in general (Thompson and Bachelard, 1970; Alexander and Penefsky, 1971), in α-globulin, increased ordered structure was induced with a concomittant decrease in the β-structure. Stevens *et al.* (1968), based on their studies on the circular dichroism of polypeptide films concluded that for the I- β-structure, the spectrum displays a positive band between 196 and 200 nm and a negative band between 216 and 220 nm and for the II-β-structure, the CD spectrum is shifted to higher wavelength and is particularly pronounced in the longer wavelength negative band at ~ 228 nm (Timasheff *et al.,* 1967). However, the contributions, if any, from the aromatic side chains should induce a positive band in the 230-250 nm region (Woody, 1978). Although α-globulin is rich in tryptophan, tyrosine and phenylalanine (Prakash, 1976; Prakash and Nandi, 1978), it is probable that the 227 nm band of α-globulin in the presence of 0.01 M Brij-36T is due to anti-parallel β-structure in the protein. However, during the curve fitting procedures, this is also evaluated in terms of α-helical and β-structures.

Figure 6 shows the near ultraviolet CD spectrum of α-globulin in 1 mM Brij-36T. There was a decrease in the amplitude of the spectrum and generation of a

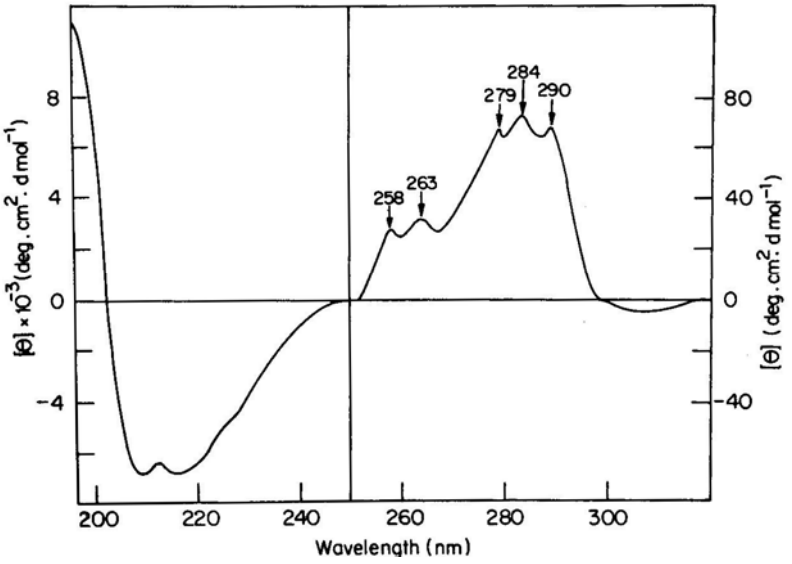

Figure 6. Circular dichroism spectra of α-globulin in 1 mM BRIJ-36T in 0.2 mM sodium phosphate buffer, pH 7.4 in the 190-320 nm region.

new CD band at 279 nm. The new CD band at 279 nm indicated a slight perturbation of the tyrosine and/or tryptophan residues at this concentration of the detergent. In most cases, the positive peaks at 264 nm and 256 nm is attributed to phenylalanine arising from (0-0) and (0-932 cm^{-1}) transitions, respectively (Strickland, 1974). It is quite possible that the blue-shifted peak at 263 nm is an indication of the perturbation of phenylalanine residue by the detergent molecules. However, as mentioned earlier, such an explanation would not rule out the CD bands arising due to the dihedral angle of disulphide, the C-S-S-bond angle and the vicinal interactions (Lindberg and Michl, 1970; Timasheff, 1970a, b; Webb *et al.,* 1973; Sears and Beyehok, 1973; and Casey and Martin, 1972).

The far ultraviolet CD spectrum of the protein in presence of 1.0 mM Brij is also shown in figure 6. The spectrum is characterized by a decrease in the negative intensity of the peak and also, the resolution of 227 nm band has decreased, probably indicating the formation of more of an aperiodic structure in the system. The curve fitting analysis of the spectrum indicates nearly 81 % aperiodic structure, 10% α-helix and 9% β-structure in the protein. At this stage, it is worthwhile noting that the protein undergoes aggregation as well as dissociation without involving tyrosine and tryptophan residues (Lakshmi and Nandi, 1978).

Figure 7 shows the near ultraviolet CD spectrum of α-globulin in 10 mM Brij-36T. In the near ultraviolet spectrum the 283 nm band becomes fairly broad with

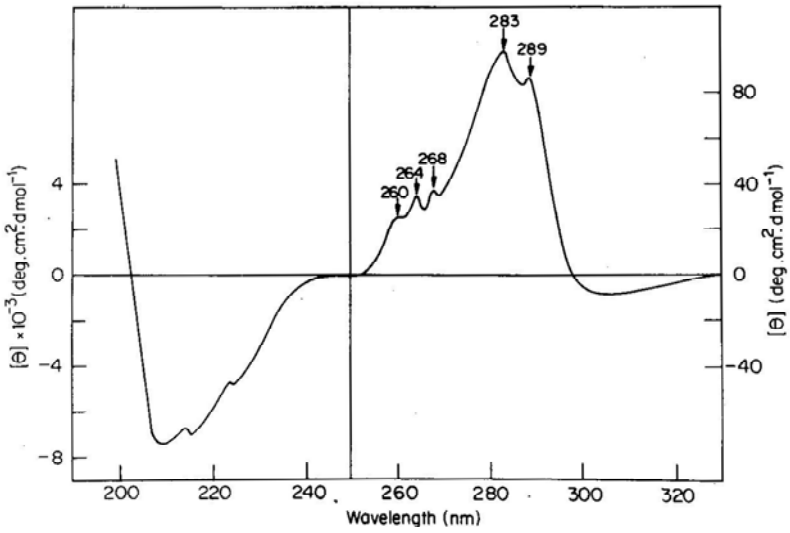

Figure 7. Circular dichroism spectra of α-globulin in 10 mM BRIJ-36T in 0.2 M sodium phosphate buffer, pH 7.4 in the 190-330 nm region.

the elimination of 279 nm band present at 1 mM Brij. The above results indicate that at this concentration of the detergent, there is probably more perturbation of the phenylalanine residue (of course with the constraint of disulphide dihedral angle and the C-S-S-bond angle) as compared to that of the tyrosine and

tryptophan residues. These results are in conformity with the already observed results of the protein at 10 mM Brij in terms of the absence of participation of tyrosine and tryptophan residues in the association-dissociation phenomenon of α-globulin (Lakshmi and Nandi, 1978).

The far ultraviolet CD spectrum is also shown in figure 7. The intensity of the spectrum remains the same as that of the CD spectrum of protein in 1 mM Brij. The CD bands at 209-210 and 227 nm become more pronounced as compared to those at 1.0 mM. The curve fitting analysis of the spectrum shows that nearly 10% of the protein structure consists of α-helix, 21% β-structure and 69% aperiodic structure indicating the formation of more of β-structure in the protein which appears to be the trend as was discussed earlier. This concentration of 10 mM Brij, is higher than the critical micellar concentration of the detergent and results have to be interpreted with much caution.

(B) Triton X-100

In figure 8 are shown the far ultraviolet circular dichroic spectra of α-globulin in presence of 10 μM, 1 and 10 mM Triton X-100, respectively. The spectrum at

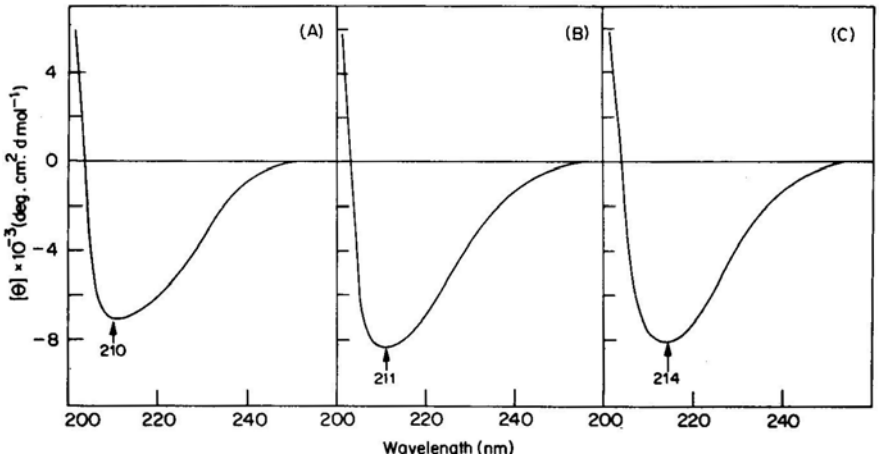

Figure 8. Far ultraviolet circular dichroic spectra of α-globulin in 10 μM, 1 and 10 mM Triton X-100 in 2.0 M sodium phosphate buffer, pH 7.4, in the 200-260 nm region.

10 mM Triton is very similar to that of the protein in buffer alone, with minimum at 210 nm; however the intensity of the spectrum is decreased. The curve fitting analysis indicates an increase in aperiodic structure to nearly 78% with a corresponding decrease in the β-structure. On the other hand, at higher concentration of Triton X-100 (1.0 mM), the percentage of aperiodic β- and α-helical structures remain the same, but the trough has a minimum at 211 nm instead of 210 nm and is broader towards larger wavelengths. However, at this concentration of Triton, the intensity of the trough is increased as compared to the 10 μM triton spectrum and is almost equal to that of the control protein in buffer alone, probably indicating the formation of more ordered structure. At still higher

concentration of Triton X-100, i.e., at 10 mM, there is a significant shift in the trough minima to 214 nm. The curve fitting analysis showed nearly 13 % α-helix, 8% β-structure and 79% aperiodic structure as indicated in table 1. Apparently even at 10 mM Triton X-100 which is above the critical micellar concentration nearly 21 % ordered structure is present in the protein. Stevens *et al.* (1968) have shown that the circular dichroic spectra of β-structured polypeptides of the category I, termed as I-β display a strong negative band between 210-220 nm very similar to the optical rotatory dispersion patterns of a number of poly-amino acids (Fasman and Potter, 1967). In view of these results the broad negative band of the protein at 214 nm which shifted from 210 nm in presence of 10 mM Triton X-100 might have arisen from the parallel β-structure or I-β structure. Obviously the broad nature of the CD band indicates that it is a combination of aperiodic, β and α-helical structures. However, the point that is relevant is that the shift of 4 nm in the protein peak from 10 mM Triton to 10 mM triton is probably due to the formation of I-β-structure in the protein in the presence of the detergent Triton X-l00.

Discussion

The circular dichroism spectra of the α-globulin under various solution conditions of cationic detergents like CTAB or nonionic detergents like Brij-36T or Triton X-100 indicate that considerable variation is exhibited in the secondary structure of α-globulin in terms of α-helical, β-structure and the aperiodic structures. Makino *et al.* (1973) have emphasised that the use of neutral detergents and the bile salt anions, cholate and deoxycholate, can often extract proteins from membranes without disruption of the native conformation or loss of biological activity in contrast to commmon synthetic ionic-detergents, notably dodecyl sulphate which ordinarily solubilise lipid-associated proteins in inactive and denatured form. The effect of the anionic detergent, SDS, on the conformation of α-globulin has been investigated in detail earlier (Prakash *et al.,* 1980). The protein unlike many of the lipid-bilayer membrane proteins, undergoes different kinds of conformational transitions in all the three kinds of detergents, viz., in anionic, cationic and neutral detergents. As reported earlier, in SDS, the protein has more ordered structure in terms of α-helix structure even at 10 mM SDS concentration (Prakash *et al.,* 1980). On the other hand, in cationic detergents like CTAB along with α-helix and aperiodic structure the protein also has different conformations like antiparallel β-structure as compared to the native protein where no such structure could be identified. At these concentrations of CTAB, Lakshmi and Nandi (1977) have observed aggregates of the protein which accompanies perturbation of the tryptophan and tyrosyl residues. On the other hand, in their studies on the effect of nonionic detergents on the physico-chemical properties α-globulin they have concluded that micelles of the detergent predominantly bind to the exposed hydrophobic surfaces of the protein subunits (Lakshmi and Nandi, 1978). Keeping in view the observed effect of CTAB, on the near ultraviolet CD spectra, which is significant, it is understandable that tyrosyl, tryptophanyl and phenylalanine residues are involved in the process. Even assuming that many groups of tryptophan, tyrosine and phenylalanine are exposed to the solvent (though

thermodynamically not feasible), the observed effect of CTAB which is quite significant cannot be explained. Hence some of these aromatic residues must be exposed to the bulk solvent as a result of conformational change of the protein. This is confirmed by the far ultraviolet circular dichoric spectra of α-globulin in presence of various concentrationsr of CTAB. In fact at lower concentrations of CTAB more of α-helix is induced in the system which decreases slightly at 10 mM SDS nearly 20% of the protein was present in the form of α-helical structure (Prakash *et al.,* 1980). These induced secondary structure of α-globulin in CTAB in fact decreases at higher detergent concentration. Mattice *et al.* (1979) have reported that the class of trimethylammonium chloride detergents are an exception to the usual ability of ionic detergents to promote formation of ordered structures in oppositely charged homopolypeptides. They have observed that the CD of fully ionized poly L-aspartic acid, is nearly unaffected by dodecylammonium chloride, whereas, the detergent destablizes the helical form of poly L-glutamic acid, such a disorganization of the tertiary structure of the protein by CTAB is expressed in a decrease of the CD bands related to the tyrosine and tryptophan chromophores and also the appearance of the vibronic fine structure in the phenylalanine band zone in α-globulin (see results). Helenium (1971) has observed that the lipid-free protein obtained with SDS or CTAB had altered immunological properties. Our observations of the effect of SDS and CTAB on the induced conformational states of α-globulin might have a bearing in explaining similar results.

However, neutral detergents have been shown to have an advantage that their availability in free concentration is automatically limited by their low critical micellar concentration, such that cooperative binding and denaturation cannot occur for any reasonable excess addition of the detergents. However, this does not exclude the possibility of a change in the conformation of protein in presence of neutral detergents possibly leading to a loss of activity or association-dissociation phenomenon in the protein. The results in the present study with Brij-36T in the near ultraviolet region indicate that the spectral perturbation of the protein cannot be directly correlated with the change in the secondary structure of the protein. In the near ultraviolet region at various concentrations of Brij-36T only the vibronic transitions of phenylalanine residue is altered significantly. At this concentration of the detergent the protein has been shown to undergo aggregation and dissociation (Lakshmi and Nandi, 1978). Looking at these results it is quite possible that some of the phenylalanine residues are in the contact areas of the subunits or are entropically trapped or released during the aggregation or dissociation of the protein respectively. In the far ultraviolet region, as compared to the ionic detergents, where there is an induced α-helix conformation, in presence of Brij-36T along with the already present α-helix region, a new CD band is observed at 227 nm which has been attributed to the II-β or anti-parallel β-structure (see results). The above result is interesting in the sense that these nonionic detergents not only are active in generating probably some α-helix structure in the system but also in generating new kinds of structures that probably were not present in the native structure in a significant amount. Probably these Π-β-structures are a pre-aggregation or pre-dissociation step leading either to aggregation or dissociation. On the other hand, in Triton X-100 even though it is also a nonionic detergent a

different CD band is observed at 214 nm which is attributed to the I-β structure more akin to the parallel β-structure. Further, Lakshmi and Nandi (1978) have observed that in Triton X-100 also the protein undergoes an association-dissociation phenomenon above cmc of the detergent. This means that the secondary structure of the protein either I-β or II-β by itself is not probably responsible for the aggregation and dissociation of the protein directly.

The analysis of the CD spectrum of α-globulin in presence of various detergents reported in this paper can serve as a good example of the serious limitations which exist in the interpretation of the circular dichroism spectra of various proteins under varying solvent conditions in terms of specific types of conformations. In particular, at present it is still a difficult task to assess quantitatively the contributions made by side-chain chromophores to the far ultraviolet circular dichroism spectra inspite of the several studies available on this subject. Further, a practical limitation also arises from the inadequacy of polypeptide models or proteins whose structures are well established as models to predict quantitatively the circular dichroism contributions from specific conformations. However, it has been possible to analyse some of the unusual features of the far ultraviolet CD spectrum of α-globulin in terms of theoretical considerations about the α-helix spectrum and the variability in terms of the contributions of β-structures specially I-β and Il-β-structures.

Acknowledgements

We would like to thank Prof. S. N. Timasheff, BiochemistryDepartment, Brandeis University, Waltham, Massachusetts, USA for allowing to use the CD equipment and the computer facilities. Our thanks are also to Prof. G. D. Fasman of Biochemistry Department, Brandeis University, Waltham, Massachusetts, USA for making some of his programmes available for the curve fitting procedures in the Digital PDP-11 computer.

References

Alexander, T. and Penefsky, H. S. (1971) in *Methods in Enzymology* ed. W. B .Jakoby, (New York: Academic Press) pp. 219-230.
Aoki, K. and Hiramatsu, K. (1974) *Anal. Biochem.,* **60,** 213.
Birdi, K. S. (1973) *Biochem J.* **135,**253.
Bornet, H. andEdelhoch, H. (1971) *J. Biol Chem.,* **246,** 1785.
Casey, J. P. and Martin, R. B. (1972) *J. Am. Chem. Soc.,* **94,** 6141.
Dunkey, P. R., Holmes, H. and Rodnight, R. (1976) *Biochem. J.* **157,** 661.
Fasman, G. D. and Potter, J. (1967) *Biochem. Biophys. Res. Commun.,* **27,** 209.
Greenfield, N. J. and Fasman, G. D. (1969) *Biochemistry,* **80,** 4108.
Helenius, A. and Simons, K. (1971) *Biochemistry,* **10,** 2542.
Helenius, A. and Simons, K. (1972) *J. Biol Chem.,* **247,** 3656.
Helenius, A. and Simons, K. (1975) *Biochem. Biophys. Acta,* **415,** 29.
Heller, J. (1968) *Biochemistry,* **7,** 2906.
Hong, K. and Hubbell, W. L. (1972) *Proc. Natl Acad. Sci., USA,* **69,** 2617.
Ikeda, K. And Hamaguchi, K. (1970) *J. Biochem.* (*Tokyo*) **68,** 785.
Jacks, T. J., Barker, R. H. and Weigang, Jr., (1973) *Int J. Peptide Protein Res.,* **5,** 289.
Jirgensons, B. (1963a) *J. Biol. Chem.,* **238,** 2716.
Jirgensons, B. (1963b) Proceedings, Seed Protein Conference, SURDV, New Orleans, Louisiana, USA, p. 252.

Jirgensons, B. (1967) *J. Biol. Chem.,* **42,** 912.
Jirgensons, B. (1980) *Makromol. Chem. Rapid Comrnun.* **1,** 97.
Koshiyama, I. (1972) *Int J. Peptide Protein Res.,* **167.**
Lakshmi, T. S. and Nandi, P. K. (1977) *Int J. Pept. Protein Res.,* **10,** 120.
Lakshmi, T. S. and Nandi, P. K. (1978) *Int J. Pept. Protein Res.,* **12,** 197.
Lederer, F. (1968) *Biochemistry,* **7,** 2169.
Lindberg and Michl, J. (1970) *J. Am. Chem. Soc.,* **92,** 2619.
Makino, S. and Tanford, C. (1973) *Fed. Proc,* **32,** 572.
Makino, S., Reynolds, J. A. and Tanford, C. (1973) *J. Biol. Chem.,* **248,** 4926.
Mattice, W. L., McCord, R. W., Shippey, P. M. (1979) *Biopolymers,* **18,** 723.
Nakaya, K., Yamada, K., Onozowa, Y. and Nakamura, Y. (1971) *Biochim. Biophys. Acta.,* **251,** 7.
Nath, R., Rao, K. H. and Giri, K. V. (1957) *J. Sci. Ind. Res.,* **16,** 228.
Nozaki, Y., Reynolds, J. A. and Tanford, C. (1974), *J. Biol. Chem.,* **249,** 4452.
Prakash, V. (1976) *Studies on the physicochemical properties of sesame* (*Sesamum indicum*) *seed protein,* Ph.D. Thesis, Mysore University, Mysore.
Prakash, V. and Nandi, P. K. (1967a) *Int. J. Pettide Protein Res.,* **8,** 385.
Prakash, V. and Nandi, P. K. (1976b) *Indian J. Biochem. and Biophys.,* **13,** 12.
Prakash, V. and Nandi, P. K. (1977a) *Int J. Peptide Protein Res.,* **9,** 319.
Prakash, V. and Nandi, P. K. (1977b) *Int J. Peptide Protein Res.,* **9,** 97.
Prakash, V. and Nandi, P. K. (1977c) *J. Biol. Chem.,* **252,** 240.
Prakash, V. and Nandi, P. K. (1978) *J. Agr. Chem., Food Chem.,* **26,** 320.
Prakash, V., Nandi, P. K. and Jirgensons, B. (1980) *Int. J. Peptide Protein Res.,* **15,** 305.
Prakash, V. (1980) Abstract No. (Biopolymers) 43 of the proceedings of the Second Congress of FAOB and Golden Jubilee Meetings of the Society of Biological Chemists, India.
Prakash, V. and Narasinga Rao, M. S. (1982) Proceedings of the first National Symposium on Biorganic Chemistry, Indian Institute of Science, Bangalore (July).
Robinson, N. C. and Capaldi, R. A. (1977) *Biochemistry,* **16,** 357.
Sears, D. and Beychok, S. (1973) in *Physical Principles and Techniques of Protein Chemistry* ed. S. Leach (New York: Academic Press) Vol. C.
Sinha, N. K. and Sen, A. (1962) *Trans. Bose. Res. Inst.,* Calcutta (India), **25,** 37.
Shukolynkov, S. A., Chizhevich, E. P., Korchagin, V. P., Osipov, V. V., Tyurin, V. A. and Fedosov, Yu, (1976), *Biokhimiya,* **41,** 61.
Strickland, E. H. (1974) *CRC Crit. Rev. Biochem.,* **2,** 113.
Stevens, L.,Townend, R. ,Timasheff, S. N., Fasman, G. D. and Potter, J. (1968) *Biochemistry,* **7,** 3717.
Thompson, M. F. and Bachelard, M. S. (1970) *Biochem J.,* **118,** 25.
Timasheff, S. N. (1970a) *Accts. Chem. Res.,* **3,** 62.
Timasheff, S. N. (1970b) in *Enzymes* ed. P. O. Boyer (New York: Academic Press) 3rd ed. Vol. 2., pp. 371
Timasheff, S. N., Susi, H., Townend, R. Stevens, L., Gorbunoff, M. J., Kumosinski, T. F. (1967) *Conformation of Biopolymers,* ed. G. N. Ramachandran (New York: Academic Press) Vol. I, p. 173.
Ushiwata, A., Nakaya, K. and Nakamura, Y. (1975) *Biochim. Acta.,* **393,** 215.
Ventura, M. M. and Lima, H. T. (1963) *Biochim. Biophys. Acta.,* **126,** 551.
Viser, L. and Blout, E. R. (1971) *Biochemistry,* **10,** 743.
Webb, J., Strickland, R. W. and Richadrson, F. S. (1973) *J. Am. Chem. Soc.,* **95,** 4775
Williams, J. G. and Gratzer, W. B. (1971) *J. Chromatog,* **57,** 121.
Woody, R. W. (1978) *Biopolymers,* **17,** 1451.