Some Conformational and Immunological Properties of a Bovine Brain Acidic Protein (S-100)*

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ABSTRACT: Antibodies to a highly acid, brain-specific protein found in vertebrates, the S-100 protein, can be obtained using the purified bovine S-100 complexed with methylated bovine serum albumin as immunogen. The presence of antigenic activity in brain extracts from a number of vertebrates measured by a quantitative complement (C′) fixation technique indicates an unusually close serological relationship among these S-100 proteins. When the purified bovine S-100 protein is heated in a Tris–saline buffer (pH 7.4) for 10 min at temperatures from 20 to 60°, the per cent C′ fixation, measured at 2–4°, gradually decreases and the antigen concentration giving maximum C′ fixation increases. After heating at 70° or higher, no antigenic activity is detected. In the presence of 1×10^{-3} M EDTA, 2-mercaptoethanol, or $CaCl_2$, the protein is protected from thermal denaturation. Divalent cations other than Ca^{2+} do not exhibit this protective effect. Studies of the optical rotatory dispersion of bovine S-100 solutions in Tris–saline buffer (pH 7.4) at various temperatures indicate that the dispersion curve displays a trough with a minimum at a wavelength of 233 mμ and that this trough value becomes less negative as the temperature increases. This may be interpreted as the destruction of α-helical structure as the temperature is increased.

Upon cooling the heated solution, the $[m']_{233\,m\mu}$, the reduced mean residue rotation at 233 mμ, reverts to the original value only if EDTA has been present, to a lesser extent in the presence of mercaptoethanol or $CaCl_2$, and only partially in Tris–saline buffer. When the protein is dissolved in distilled water, optical rotatory dispersion measurements show that at 20°, the optical rotatory dispersion curve has a maximum [m′] of 29,500 at 198 mμ and a minimum of about −6000 at 233 mμ. Among computer generated optical rotatory dispersion curves representing varying proportions of α helix, β, and random chain, the one with 40% α helix, 30% β, and 30% random structure most closely resembles bovine S-100 in distilled water at 20°. As the protein solution is heated the $[m']_{198\,m\mu}$ value decreases and the $[m']_{233\,m\mu}$ becomes less levorotatory, indicating destruction of the α helix. However, with the rise in temperature, a peak gradually appears at about 190 mμ. This is interpreted as the formation of a new protein conformation at high temperature, perhaps the parallel β structure. Solutions which have been heated to intermediate temperatures and then cooled retain a small amount of the 190-mμ peak and partially regain the 198-mμ peak. After 90° incubation, the cooled protein retains none of the $[m']_{190\,m\mu}$ peak, but reverts to about 25% α helix.

The presence of a highly acid protein fraction unique to vertebrate brain was first reported by Moore and McGregor (1965) on the basis of electrophoretic studies. Moore (1965) examined the protein purified from bovine and rabbit brain and found that its acidity could be accounted for by the presence of about 30 mol % acidic amino acid residues, mostly charged at neutral pH and lesser amounts of basic amino acids. The protein was named "S-100" on the basis of its solubility in 100% saturated ammonium sulfate. The molecular weight of the bovine protein was estimated to be 30,000 by Moore (1965).

Moore (1965) found that the electrophoretic properties and the amino acid composition of rabbit brain S-100 and bovine brain S-100 were very similar. We thought a study of the antigenic relationship of the S-100 proteins from different species would give further information about the structural similarity of the molecules. Using the quantitative complement (C′) fixation technique of Wasserman and Levine (1961) we report experiments here indicating that not only are rabbit and bovine brain S-100 antigenically similar, but a close relationship exists in this brain protein among a wide variety of vertebrates. The C′ fixation technique has been used in the past to study conformational changes in proteins during physical and chemical treatments (Van Vunakis and Levine, 1963; Gerstein *et al.*, 1963, 1964). In the experiments described here, we have found changes in the antigenicity of bovine S-100 during thermal denaturation. By studying conditions that protect the molecule against this denatura-

* Publication No. 548 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received October 9, 1967.* Supported in part by research grants from the National Institutes of Health (AI-01940 and AM-05852), National Science Foundation (GB-5576), and the National Aeronautics and Space Administration (NSG-375).

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tion and by relating the antigenic changes in the molecule with changes in the optical rotatory dispersion during heating, we have obtained some information regarding the conformation of the native and the denatured protein.

Materials and Methods

Protein. The S-100 brain protein studied in these experiments was purified by Moore (1965) from bovine brain, and was kindly supplied by Dr. Blake Moore.

Antiserum. Antibodies to bovine brain S-100 were successfully produced in rabbits after complexing the purified protein with methylated bovine serum albumin according to the method of Plescia *et al.* (1964) and using this complex as immunogen. The antiserum gave one major band in double-diffusion tests in agar with purified beef brain S-100 protein as antigen and, in addition, a second minor band with crude brain extract as antigen.

C′ Fixation. Reagents and procedures for the quantitative C′ fixation technique have been described by Wasserman and Levine (1961). For the experiments involving heat denaturation, the S-100 protein was weighed, dissolved in a solution of 0.85% sodium chloride at a concentration of 1 mg/ml, and stored frozen. Just before the heat treatment, the solution was diluted to 10 μg/ml with Tris–saline buffer (0.01 M Tris–0.14 M sodium chloride, pH 7.4), or in the same buffer in the presence of the reagents to be tested for thermal stabilization of S-100. A portion of the solution was then heated in a water bath for 10 min at the desired temperature, quickly cooled in an ice bath, and diluted to a concentration suitable for use in the C′ fixation technique (1 μg/ml).

Optical Rotatory Dispersion. A Cary 60 recording spectropolarimeter with slit width programmed to maintain 15-Å half-band width was used for optical rotatory dispersion measurements. When the S-100 protein was heated in solutions containing the Tris–saline buffer, the optical rotatory dispersion was examined using a fused-quartz optical cell (path length, 1 cm) enclosed in a water jacket. Aliquots of a stock solution of 1 mg/ml of S-100 in 0.85% sodium chloride were diluted with cold Tris–saline buffer, or with the buffer at the same pH after the addition of calcium chloride, EDTA, or 2-mercaptoethanol. Nitrogen determinations of identical aliquots of the stock solution diluted in a similar manner with distilled water were made by a Nessler micro-Kjeldahl nitrogen analysis (Lang, 1958). The protein concentration in these solutions was 0.012%, assuming 3878 g of nitrogen/26,525 g of protein (Moore, 1965). Each protein solution was introduced to the cell, warmed to 20° for 10 min, and the optical rotatory dispersion from a wavelength of 325 to 225 mμ was determined. Subsequent optical rotatory dispersion measurements of the solution in the cell were taken after 10-min equilibration at 10° temperature increments to 90°. The solution was then cooled to 20° in the cell and another optical rotatory dispersion measurement was taken. Variation in the appropriate solvent base line caused by moving the optical cell or by temperature changes were not significant.

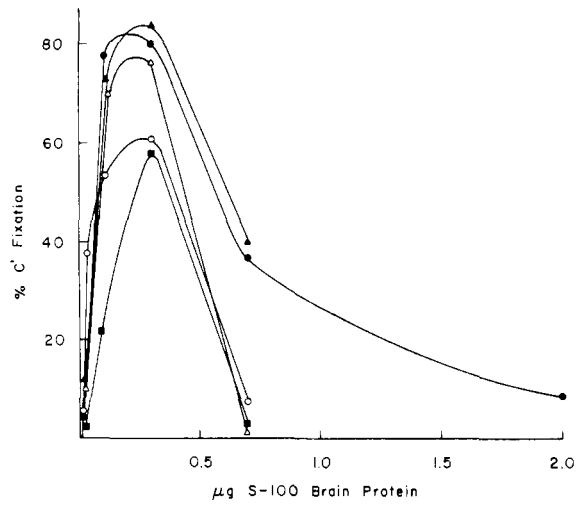

FIGURE 1: Fixation of C′ by S-100 protein (●); brain extracts from beef (▲), sheep (△), guinea pig (○), and mouse (■) with anti-S-100.

A 1-mm path-length, fused-quartz optical cell with water jacket was used for measuring the optical rotatory dispersion of solutions of the protein in distilled water from a wavelength of 324 to 185 mμ. The S-100 protein was dissolved in glass-distilled water at 5°, filtered through Millipore filter, type HA (0.45-μ pore size), and either frozen or used immediately. Protein concentration, determined by Nessler nitrogen analysis (Lang, 1958), was found to be 0.014%. Portions of this protein solution were held in the optical cell for 10 min at the desired temperature (5–90°). One solution, however, was heated for 5 hr at 90° before introduction into the cell. Optical rotatory dispersion measurements were then begun, ending about 45 min later. Afterwards, these same solutions were withdrawn from the cell, kept at room temperature for a short time, and frozen. Optical rotatory dispersion measurements at 21° were later made on these solutions. The optical rotatory dispersion of glass-distilled water from 325 to 185 mμ was taken as the reference solution. There were only minor fluctuations in base-line values after changes in water-jacket temperature or after moving the optical cell.

Data are reported as $[m']_\lambda$, the reduced mean residue rotation (Fasman, 1963). A value of 113 was taken for the mean amino acid residue weight (Moore, 1965).

Results

C′ Fixation, Species Comparison. The C′ fixation curves obtained with antiserum to the purified bovine brain protein and brain extracts from several animals are shown in Figure 1, at antiserum dilutions of 1:4000. Since the absolute content of S-100 in each extract is not known, the maximum fixation for each

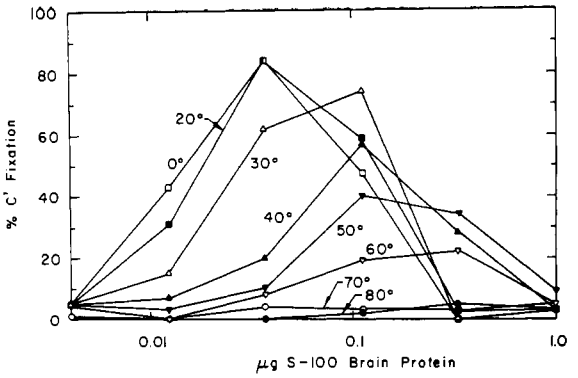

FIGURE 2: Fixation of C′ by S-100 protein after incubation (10 μg/ml of Tris–saline buffer) for 10 min at various temperatures.

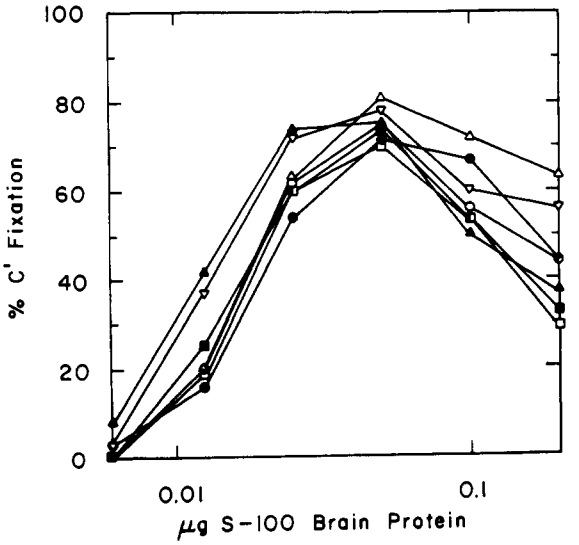

FIGURE 3: Fixation of C′ by S-100 protein after incubation (10 μg/ml) at various temperatures in Tris–saline buffer containing 1×10^{-3} M 2-mercaptoethanol: 20 (■), 40 (□), 60 (▲), 70 (▽), 80 (△), and 100° (●).

extract is plotted at the point of maximum fixation for the purified bovine S-100 with this antiserum: 0.2 μg of antigen. The index of dissimilarity, *i.e.*, the factor by which the antiserum concentration must be raised in order that a particular brain extract give maximum C′ fixation equal to that given by the homologous bovine brain protein (Wilson *et al.*, 1964), among 11 species is shown in Table I. While there are quantitative differences in the brain extract antigens among the different species, these differences are extremely small as compared to similar data obtained with other protein immune systems (Wilson *et al.*, 1964). Thus, the S-100 proteins from a large number of vertebrates appear to be closely related.

C′ Fixation, Thermal Denaturation. Portions of a solution of S-100 beef brain protein at a concentration of 10 μg/ml in Tris–saline buffer, heated for 10 min at different temperatures and then immediately cooled and diluted, give the series of C′ fixation curves shown in Figure 2. With increasing temperature there is a progressive shift in these curves toward the region of higher antigen concentration accompanied by a decrease in peak height. After incubation at a temperature of 70° or higher, no reactivity with homologous antiserum can be detected. The same series of curves is obtained when S-100 is heated at a concentration of 150 μg/ml and diluted after cooling.

The effects of various agents on the thermal denaturation of S-100 as measured serologically were studied. If S-100 is placed in Tris–saline buffer in the presence of 1×10^{-3} M 2-mercaptoethanol, the C′ fixation curves shown in Figure 3 are obtained after heat treatment. No change in the per cent C′ fixation or in the amount of antigen needed for maximal C′ fixation occurs after 10-min heat incubations at temperatures of 20, 40, 50, and 60°. Little change occurs even after heating at 70, 80, and 100°. This is in striking contrast to the C′ fixation curves in Figure 2, where the per cent C′ fixation progressively declines as the incubation temperature is increased. Several other reagents are found to protect S-100 from thermal denaturation, as measured by C′ fixation. When EDTA at a concentration of 1×10^{-3} M or more is added to the Tris–saline buffer, the C′ fixation curves using antigen heated in this solution are very similar to those in which mercaptoethanol is present. Also, calcium chloride at the same concentration in the Tris–saline buffer protects S-100, although in this case the protection is not so complete as that furnished by EDTA or mercaptoethanol. This is shown in Figure 4 which summarizes the protection of S-100 to thermal denaturation given by mercaptoethanol, EDTA, and calcium chloride in the Tris–saline buffer compared with the protein in buffer alone. From the C′ fixation curves of these four solutions at different temperatures, only the values at 0.05 μg of S-100 have been considered

TABLE I: Complement Fixation with Antiserum to Beef Brain S-100 Protein.

Brain Extracts	Rel Antiserum Concn Required for 50% Fixation	Brain Extracts	Rel Antiserum Concn Required for 50% Fixation
Beef	1.0	Pig	1.8
Rat	1.0	Rabbit	1.9
Sheep	1.0	Human	2.0
Guinea pig	1.2	Chicken	2.2
Mouse	1.3	Pigeon	2.3
		Bullfrog	2.5

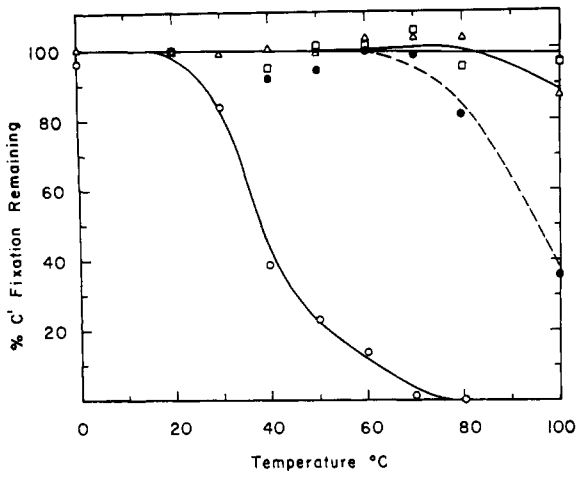

FIGURE 4: Protection of S-100 protein to thermal denaturation as measured by serologic activity. Temperature-serologic activity profile in the presence of Tris–saline buffer (○) and Tris–saline buffer containing 1×10^{-3} M 2-mercaptoethanol (△) 1×10^{-3} M EDTA (□) and 1×10^{-3} M $CaCl_2$ (●).

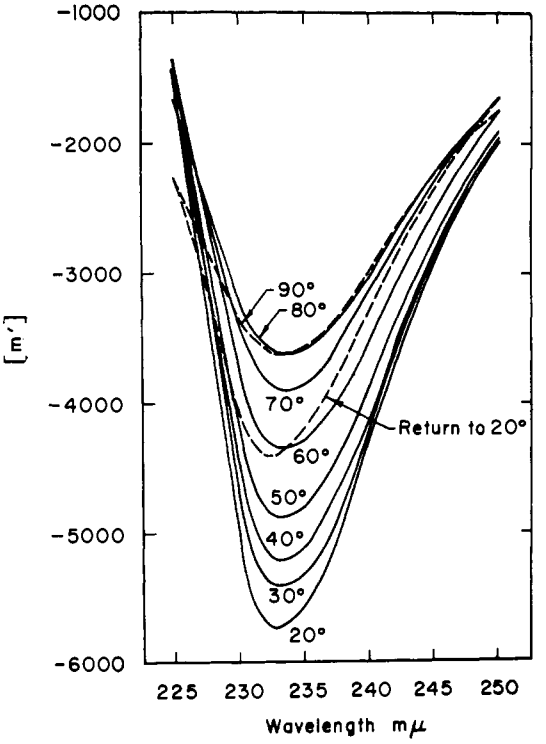

FIGURE 5: The optical rotatory dispersion of a solution of bovine S-100 protein in 0.01 M Tris–0.14 M NaCl (pH 7.4) at 20 through 90° and after cooling the 90° solution to 20°.

for Figure 4. The fraction of the 20° value remaining in each solution as the temperature increases has been plotted as C′ fixation remaining.

Although calcium chloride partially protects S-100 from thermal denaturation, salts of other divalent cations do not have this effect. At a concentration of 1×10^{-3} M strontium chloride, magnesium chloride, or barium chloride during heating in the S-100 solution, C′ fixation curves are the same as those obtained with the protein in Tris–saline alone. Cupric chloride, manganous chloride, *p*-mercuribenzoate, and *N*-ethylmaleimide at the same concentration also do not alter thermal denaturation.

Optical Rotatory Dispersion. We turned to optical rotatory dispersion studies of the thermal denaturation of bovine S-100 with the hope that the structural changes detected by C′ fixation could be better explained in terms of protein conformation. In Figure 5 is given a series of optical rotatory dispersion curves from wavelength 250 to 225 mμ obtained when an aliquot of the brain protein in the Tris–saline buffer is heated successively from 20 to 90°, then returned to 20°. Note that the 20° optical rotatory dispersion curve at a wavelength of 233 mμ has an [*m*′] value of about −5700, and that this trough at 233 mμ becomes less negative as the protein solution is heated. At 80 and 90°, the $[m']_{233\,m\mu}$ seems to reach a constant value of about −3600. The negative minimum [*m*′] at 233 mμ in the 20° curve may be taken as an indication of some α-helical content (Simmons *et al.*, 1961; Yang and McCabe, 1965; Sage and Fasman, 1966), although a quantitative estimate cannot be given because the β structure also displays a Cotton effect in this region (Davidson and Fasman, 1967; Davidson *et al.*, 1966; Sarkar and Doty, 1966). Change in the 233-mμ value during heating, as shown in Figure 5, is probably correlated with disruption of bonds important in maintaining the helical conformation (Brandts, 1964). The optical rotatory dispersion curves for S-100 show no race of additional Cotton effects from 250 to 325 mμ. Thus, the [*m*′] values at 233 mμ are not influenced by additional rotation often associated with aromatic amino acid side chains or disulfide bonds (Fasman *et al.*, 1964; Beychok, 1965; Rosenberg, 1966). When the protein solution is cooled from 90 to 20°, the $[m']_{233\,m\mu}$ again becomes more negative but never reaches the original 20° value. Apparently some, but not all, of the native helical structure is regained upon cooling the protein.

A series of optical rotatory dispersion curves can also be obtained when S-100 in Tris–saline buffer is heated in the presence of mercaptoethanol, EDTA, or calcium chloride. At 20°, the optical rotatory dispersion curve from 325 to 225 mμ with each of these solutions is the same as that obtained with S-100 in Tris–saline buffer alone. However, the change of $[m']_{233\,m\mu}$ during heating is different from the change of the protein in buffer alone (Figure 6). At temperatures from 30 to 80°, the $[m']_{233\,m\mu}$ becomes less negative, but for each temperature there is less change in the presence of mercaptoethanol, EDTA, or calcium chloride than in their absence. This can be interpreted as some protection by these agents of the helical conformation from

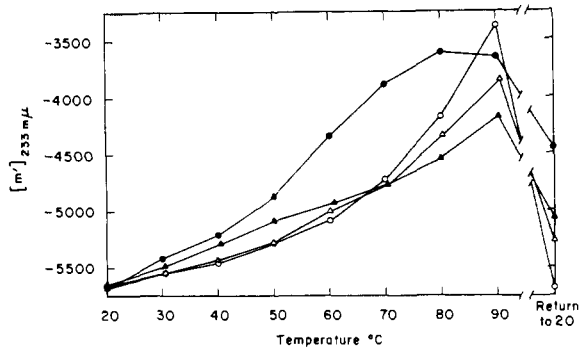

FIGURE 6: Reduced mean residue rotation, $[m']_{233}$, of bovine S-100 protein (pH 7.4) in 0.01 M Tris–0.14 M NaCl with no additional reagents (●), 1×10^{-3} M EDTA (○), 1×10^{-3} M 2-mercaptoethanol (△), and 1×10^{-2} M $CaCl_2$ (▲) at temperatures from 20 to 90°, and then returned to 20°.

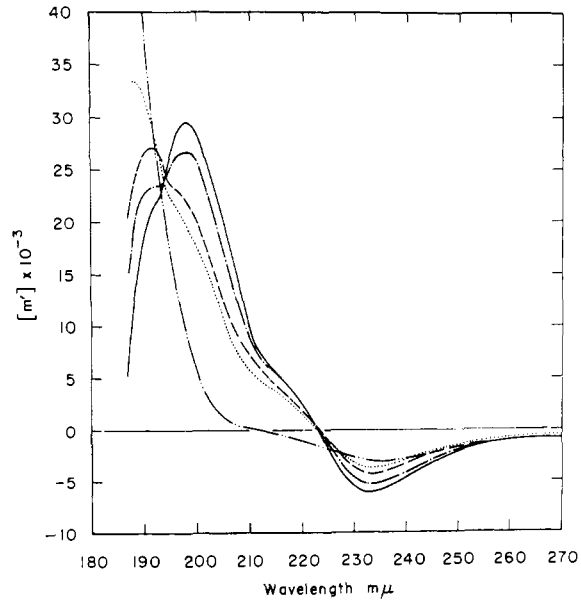

FIGURE 7: The optical rotatory dispersion of bovine S-100 protein in distilled water (pH 6.9–7.1) at 20° (——), 40° (— · —), 60° (- - - -), 70° (· · · · ·), and 90° (— · · —). The solution at 90° was held for 5 hr at that temperature before measuring, while the rest were held for 10 min before measurements began.

thermal disruption. By 90°, the $[m']_{233\,m\mu}$ for S-100 in the presence of EDTA has increased to that of S-100 in buffer alone, although mercaptoethanol and calcium chloride still give some protection. However, when the solutions are cooled back to 20°, the effect of the added reagents becomes more striking. The $[m']$ at 233 mμ in the presence of EDTA has now returned to the original 20° value. This return is not so complete in the presence of mercaptoethanol or calcium chloride, but all three reagents clearly permit a better recovery of the helical portion of the molecule upon cooling than the Tris–saline buffer alone. This suggests that the heated molecule in Tris–saline buffer alone may not only have lost some α-helical content, but may have gained a new conformation that prevents a complete return to the original structure upon cooling.

To examine this possibility, we performed optical rotatory dispersion measurements of the bovine S-100 protein in the 325–185-mμ range at various temperatures. Good evidence of structural changes can be obtained from these optical rotatory dispersion patterns and their analysis can be attempted (Greenfield *et al.* 1967). These experiments were done with S-100 in distilled water at pH 6.9–7.1 without buffer or salts to minimize the problem of absorption in the low-wavelength region of the spectrum. Aliquots of the protein solution were placed in the jacketed optical cell at the desired temperature for 10 min after which the optical rotatory dispersion measurements were begun. The resulting optical rotatory dispersion curves are shown in Figure 7. The $[m']$ of the 20° optical rotatory dispersion curve starting at a wavelength of 325 mμ (not shown) is slightly negative, gradually becoming more negative through 270 mμ and reaches a minimum value of about −6000 at 233 mμ. Then the $[m']$ steadily increases, becoming positive at about 223 mμ, exhibiting a slight shoulder around 215 mμ, and finally reaches a peak value of about 29,500 at 198 mμ. The $[m']$ then decreases with a shoulder around 192 mμ, and continues to decrease through 186 or 187 mμ when quantitative measurements become difficult.

We compared this optical rotatory dispersion curve of bovine S-100 in distilled water at 20° with the computed optical rotatory dispersion curves of Greenfield *et al.* (1967), representing different proportions of α helix, β structure, and random configuration. The shape of the S-100 curve, especially the negative trough value at $[m']$ at 233 mμ, the shoulder at 215 mμ, and the positive peak at 198 mμ is characteristic of α helix, but the absolute values are not so large as those expected for 100% α helix. The 100% α helical curve also lacks the shoulder at 192 mμ. In fact, the computed curve most similar to that obtained here with S-100 at 20° is composed of about 40% α helix, 30% β structure, and 30% random structure.

When the brain protein is heated to 40°, the shoulder at about 192 mμ (Figure 7) becomes more noticeable, and at 60°, a new peak is present at this wavelength. At 70°, this new peak has increased in magnitude and has shifted slightly to a lower wavelength. If the protein solution is heated to 90° and the optical rotatory dispersion measurement is begun after 10 min (not shown) or after 5 hr (Figure 7), the $[m']$ in this region is even larger but the exact position of the peak cannot be ascertained. On the other hand, the peak at 198 mμ gradually diminishes as the temperature of the solution increases, and finally, at 90°, is no longer visible even as a small shoulder. The $[m']_{233\,m\mu}$ becomes less negative as the temperature rises, but never reaches the value associated with a random structure (−2000)

(Sage and Fasman, 1966). The crossover wavelength, at which the $[m']$ changes in sign, remains at about 223 mμ as the temperature is raised until 90° when a shift occurs to a lower wavelength, indicating some further change in conformation at that temperature.

The decrease of $[m']$ at 198 mμ with increasing temperature almost certainly corresponds to the melting out of α-helical structure. At 90°, when no peak or shoulder at 198 mμ is visible, the α helix is probably completely destroyed. The change of $[m']_{233\,m\mu}$ toward less negative values is also an indication of the breakdown of α helix. The formation of the new peak at about 190 mμ as the temperature increases is not so readily interpretable. A peak at this wavelength is not associated with any commonly known conformation of polypeptide or protein (Greenfield *et al.*, 1967), and must therefore represent a conformation not previously studied by optical rotatory dispersion.

The heated solutions of S-100 in distilled water whose optical rotatory dispersion is recorded in Figure 7 were cooled to room temperature, stored by freezing, and later examined at 21° for changes in the optical rotatory dispersion curves. The optical rotatory dispersion curves at 21° of the S-100 solution cooled from 40, 70, and 90° are shown in Figure 8, together with the original 20° curve for comparison. The peak at 190 mμ is considerably diminished in all cases but is still evident in the solution heated at 70° before cooling and to a lesser extent in the 40° solution. Both of these solutions also exhibit a return of some of the 198-mμ peak, so that the region between 190 and 200 mμ superficially appears to be a single broad peak. Apparently, the S-100 molecule heated to 40 or 70° regains some α-helical structure upon cooling, but also retains a portion of the new conformation which had appeared during heating. On the other hand, the solution heated at 90° for 5 hr does not exhibit the 190-mμ peak after being cooled, further indicating that another different structure was produced. However, the original α-helical structure only partly reappears in the cooled protein, since the $[m']_{198\,m\mu}$ value is quite low. Of the computer generated curves given by Greenfield *et al.* (1967), the closest fit to the optical rotatory dispersion curve of the cooled 90° solution is that of a polypeptide with about 25% α helix, 25% β structure, and 50% random structure.

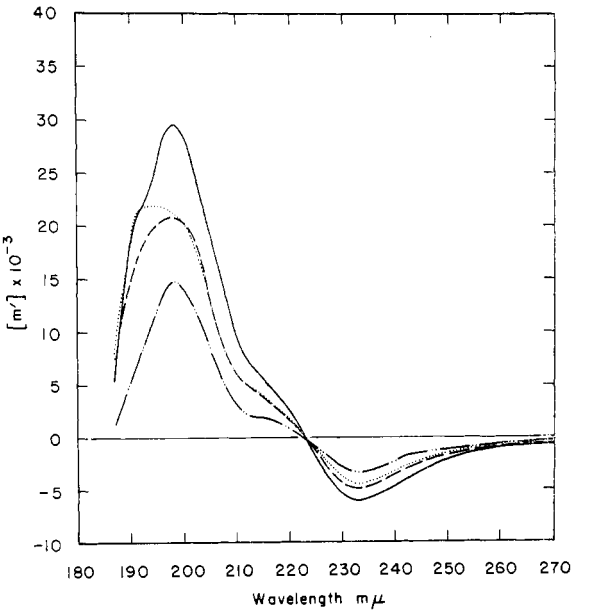

FIGURE 8: The optical rotatory dispersion at 21° of some of the same solutions of bovine S-100 protein shown in Figure 7 after being heated at 40° (- - -), 70° (· · · · ·), and 90° (— · · —). The optical rotatory dispersion of unheated bovine S-100 protein at 20° (——) is shown for comparison.

Discussion

The gradual appearance of a peak at a wavelength of about 190 mμ during thermal denaturation of S-100 protein must be due to the formation of an unusual polypeptide conformation. None of the usual structures associated with polypeptide conformation has an optical rotatory dispersion curve with a maximum $[m']$ at 190 mμ (Greenfield *et al.*, 1967). For instance, the α-helical structure is known to have a peak at about 198 mμ. Such a peak can be seen in the optical rotatory dispersion curves of the S-100 solutions along with the 190-mμ peak at intermediate temperatures. Therefore, the 190-mμ peak does not seem to be associated with the α helix. The β conformation, probably the antiparallel β, formed by hydrogen bonds between CO and NH groups on two polypeptide chains lying side by side but with reverse chain direction, has a peak at a wavelength of about 205 mμ (Davidson and Fasman, 1967), too far removed to account for a peak at 190 mμ. The random conformation has been found to have a positive $[m']$ value at 190 mμ, but the actual maximum is further into the ultraviolet region of the spectrum. Also, the optical rotatory dispersion curve of the random structure has a large negative trough at about 205 mμ. Even at 90° the S-100 solution has a positive $[m']_{205\,m\mu}$ value rather than a negative value. The optical rotatory dispersion curve at 90° would indicate that a third structure has formed. This seems to eliminate the random conformation as a possible explanation for the 190-mμ peak. Pysh (1966) has calculated the circular dichroism properties for the antiparallel β conformation and also the parallel β conformation in which polypeptide chains lie side by side with parallel chain direction. Of these two β structures, only the antiparallel β has been partially identified and studied by optical rotatory dispersion. If the theoretical circular dichroism properties suggested by Pysh (1966) are converted into optical rotatory dispersion curves (N. M. Tooney, unpublished data), the parallel β conformation would be expected to have a large positive peak at about 190 mμ. The optical rotatory dispersion data obtained by us with the heated S-100 solutions can therefore tentatively be interpreted

as indicating a gradual increase of parallel β structure with the rise in temperature to 90°. Above this temperature another structure is formed. Unequivocal assignment of the parallel β structure to the 190-mμ peak in the optical rotatory dispersion peak of heated S-100 can only come, of course, after further studies with other techniques. The shoulder at 192 mμ in the unheated sample, probably is indicative of some β structure in the native conformation.

The information obtained by studying the optical rotatory dispersion of S-100 in solution provides a basis for explaining the nature of the changes in the structure of S-100 as followed by C′ fixation. In the latter technique the antigenicity of the heated protein solution was assayed only after cooling to 0° and dilution to 1 μg/ml. Therefore, the best comparison of the two techniques is between the optical rotatory dispersion curves of the S-100 solutions cooled after being heated and the C′ fixation data. The lack of antigenicity of the S-100 protein in solutions heated at 70–100° may be related somehow to the loss of α-helix content of the cooled molecule since the corresponding $[m']$ at 233 and 198 mμ do not return completely to the original values. This can be better illustrated in the data shown in Figure 6, where it can be seen that the presence of EDTA does not prevent the loss of α helix, indicated by the decrease in magnitude of $[m']_{233\,m\mu}$ trough at 90°, while upon cooling, the original $[m']_{233\,m\mu}$ is recovered. Similarly, the heated protein solution in EDTA has lost little antigenicity after cooling (Figure 4). EDTA and the other protecting agents may either inhibit the formation of the new structure (parallel β conformation) at high temperature or destabilize the new structure upon cooling, rather than preventing the destruction of α helix. This might allow the α helix to revert completely to the native state. The observation that the highly negatively charged S-100 protein maintains a highly ordered structure (40% α, 30% β, and 30% random chain) at pH 7.4, while negatively charged polypeptides usually assume the random conformation (Sage and Fasman, 1966; Doty *et al.*, 1957) strongly suggests that other bonds (*e.g.*, hydrophobic) probably play an important role in maintaining the native conformation.

Variations in serological activity associated with evolutionary differences have been seen with hemoglobins, lactic dehydrogenases, triosephosphate dehydrogenases, glutamic dehydrogenases, and aldolases (Wilson *et al.*, 1964) and with many serum proteins (Goodman, 1962). However, we have demonstrated here that S-100 proteins lack large species differences. If this finding indicates a similarity in structure of S-100 proteins throughout the vertebrates, then the possibility of a functional similarity is not remote. Unfortunately, the function of the S-100 protein in the brain is not yet fully known. De Robertis (1967) has shown that antiserum to S-100 has a detrimental effect on nerve stimulus transmission and results in ultrastructural changes in nervous tissue. The S-100 protein in rats exhibits a rapid metabolic turnover *in vivo* (McEwen and Hyden, 1966) and seems to be located in the cytoplasm of glial cells and in nuclei of neurons (Hyden and McEwen, 1966). The relationship of these findings and those reported in this paper to the function of this unique acid protein in vertebrate brain remains to be elucidated.

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