Optical Rotatory Dispersion and the Conformation of Human γ -Globulin

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Extensive structural studies on γ -globulin have failed to provide a satisfactory model of the molecular geometry that will account for all the experimental findings. Porter's (1962) work on the fractionation of the protein after papain digestion, and the results obtained by Edelman & Poulik (1961) and Edelman & Benacerraf (1962) on reduction, have led to a better understanding of the basic structure; that is the number and size of the chains and the areas of the molecule involved in specific antigen-antibody reactions. However, available data still leave an equivocal picture of the secondary structure and of the general conformation of the γ -globulins.

The technique of optical rotatory dispersion has in recent years been applied to the study of the structure of human γ -globulin (Jirgensons, 1957, 1958*a, b*, 1960, 1962; Imahori & Momoi, 1962), of rabbit γ -globulin (Winkler & Doty, 1961) and of bovine γ -globulin (Tanford, Buckley, De & Lively, 1962).

The results show that these globulins have abnormal optical rotatory properties. These have been interpreted as indicating the complete lack of helical conformation; the magnitude of the deuterium-hydrogen exchange reaction (Blout, de Lozé & Asadourian, 1961) and the high content of 'helix-resisting' amino acids, particularly proline, have also been cited as evidence for this contention (Winkler & Doty, 1961). The demonstration that helix formation can be induced with 2-chloroethanol (Winkler & Doty, 1961; Imahori & Momoi, 1962) renders it unlikely that the amino acid composition is the factor limiting helix formation. If helix is absent as suggested, the changes observed could be due to an effect of the solvent on the conformation of the protein, involving changes in hydrophobic bonding similar to those suggested by Tanford, De & Taggart (1960) for β -lactoglobulin.

Schellman & Schellman (1961) and Imahori & Momoi (1962) put forward a different interpretation of the anomalous results. They indicate that the results can be explained on the basis of periodic structure within the molecule and suggest that mixtures of right-handed α -helix and β -form would give such results. Kushner (1961) has interpreted Tanford's data on β -lactoglobulin similarly.

Since our optical rotatory dispersion studies on human serum albumin (Callaghan & Martin, 1962)

we have carried out a similar series of experiments on human γ -globulin. We hoped that if the molecule did contain two structures one would be more stable than the other and that it would prove possible to delete the less stable conformation and demonstrate the presence of the other.

EXPERIMENTAL

Materials

Six batches of human γ -globulin have been studied. The LKG samples (batch nos. 44, 49; Lister Institute, Elstree) were 10% solutions in 0.15M-NaCl. The EG samples (batch nos. 105, 111 and 137; Lister Institute, Elstree) were freeze-dried. These five samples were prepared by the ether-fractionation technique (Kekwick & Mackay, 1954). The sixth sample was an American preparation (no. 1979, Squibb and Sons Ltd., New York), prepared by the ethanol-fractionation technique (Cohn, Strong, Hughes & Milford, 1946) and freeze-dried. 2-Chloroethanol was twice fractionally distilled (b.p. 128–129°). Urea and all inorganic salts were A.R. grade.

Methods

Moving-boundary electrophoresis. Samples were dialysed to equilibrium at 2° against sodium phosphate buffer, pH 8.0 and *I* 0.2, and diluted with buffer to a concentration of 1.5% of protein. Measurements were made in the Tiselius apparatus at 0° with a potential gradient of 6 v/cm. Optical observations, by the diagonal-schlieren method (Philpot, 1938), were recorded photographically with light at 546 m μ .

Ultracentrifugal analysis. Dialysed samples were diluted to a 1% protein concentration so that the solution contained phosphate buffer, pH 8.0 and *I* 0.2, in 0.15M-NaCl. The solutions, in 12 mm. cells, were subjected to 250000*g* in the Svedberg oil-turbine centrifuge. The optical-recording technique was the same as used for electrophoresis.

Measurement of pH. This was done with a glass electrode and calomel half-cell (with liquid-liquid junction).

Immuno-electrophoresis. Samples were subjected to electrophoresis in agar (Scheidegger, 1955) and tested against antisera prepared against normal human serum proteins in rabbits (Weitz, 1952).

Immunochemistry. Native and dialysed samples were tested against specific antisera to normal human γ -globulin, prepared in rabbits, by the agar-plate technique (Elek, 1949; Ouchterlony, 1949).

Dialysis. Samples were dialysed for 3 days against frequent changes of 0.15M-NaCl or distilled water.

Chemical estimations. Total hexose was measured by the method of Sørensen & Haugaard (1933). Sialic acid was measured by the method of Svennerholm (1957, 1958).

Nitrogen was measured by the Kjeldahl method with a nitrogen-protein conversion factor of 6.25.

Dispersion methods. Dispersion measurements were made on the spectropolarimeter described by Callaghan & Martin (1962). Measurements were made at intervals between 350 and 650 $m\mu$. A 1 dm. cell was used in all cases.

Solutions for optical measurements were prepared under standard conditions by dilution of a 5% stock solution of γ -globulin in 0.15M-NaCl. Each solution was kept at room temperature for at least 12 hr. Nitrogen was determined on this solution.

Interpretation of results

The interpretation of the optical rotatory dispersion data has been discussed by Callaghan & Martin (1962). Calculations based on the Drude equation are from rotations corrected for the refractive index of the solvent, and are related to the aqueous solution, not to vacuum. In the calculations with the Moffitt equation (Moffitt & Yang, 1956) the mean residue weight of γ -globulin is taken as 113.5 (Brand & Edsall, 1947); λ_0 212 $m\mu$ was found to be most satisfactory for native γ -globulin.

RESULTS

The results used in assessing the purity of the γ -globulin samples studied are shown in Table 1.

Alterations in the protein concentrations within the range 0.2–1.5 g./100 ml. produce no change in the rotatory dispersion, but at low concentrations errors occur owing to the small measured rotations. As with albumin, it is most convenient and econ-

omical to work with approximately 0.5 g. of protein/100 ml. of solution.

Variations in sodium chloride concentration at pH 7.0 from 0.1 to 1M cause no change in the rotatory properties.

Table 2 lists the dispersion data for the γ -globulin samples (dissolved in 0.15M-sodium chloride). With due regard to the inherent differences in the samples, the overall agreement is reasonable and that between the purest samples is close.

The significant points in this Table are the low λ_c values (cf. albumin; Callaghan & Martin, 1962) associated with a relatively positive laevorotation and a b_0 of zero. It is these atypical values that have led to the view that γ -globulin is non-helical.

The effects of pH change in the range 1–12 are shown in Fig. 1 and Table 3. The results are similar to those of Jirgensons (1954, 1957), the rotation remaining almost constant over the range 4–10. At extremes of pH the laevorotation increases rapidly, indicating molecular unfolding. Paradoxically, b_0 becomes negative, suggesting an increase in the extent of folding of the molecule. A similar paradox is observed in the presence of urea. Throughout this study, measurements have been made over the horizontal portion of the pH curve.

The action of urea on γ -globulin is time-dependent at all concentrations, and, depending on the sample, a maximum rotation is reached after

Table 1. *Analytical data on human γ -globulins examined*

For details of samples see Experimental section.

		LKG sample		EG sample			Squibb sample no. 1979
		44	49	105	111	137	
Electrophoretic analysis (content expressed as % of total protein)	Alb.	3.9	7.1	—	0.7	—	—
	$\alpha + \beta$	6.3	6.5	2.5	4.3	—	3.0
	γ_1	11.9	10.5	5.0	9.1	3.9	7.0
	γ_2	77.9	77.9	91.0	86.0	96.1	90.0
Ultracentrifugal analysis (content expressed as % of total protein)	4.5 s	3.0	2.0	—	—	—	—
	7 s	82.6	86.0	100	100	100	100
	10 s	14.4	11.0	—	—	—	Trace
	19 s	Trace	1.0	—	—	—	—
Sialic acid content (mg./100 mg.)		0.4	0.49	0.23	0.23	0.32	0.1
Hexose content (mg./100 mg.)		1.26	1.26	1.31	1.14	1.24	1.2

Table 2. *Optical-rotation and dispersion data for human γ -globulin*

Samples were dissolved in 0.15M-NaCl at 20° at a concentration of approx. 0.5 g./100 ml. K and λ_c are derived from the Drude equation, a_0 and b_0 from the Moffitt equation.

Sample and batch no.	$-\alpha_D$	$-\alpha_{500 m\mu}$	$-10^{-6} K$	$\lambda_c (m\mu)$	$-a_0$	$-b_0$
LKG 44	52.1°	76.7°	15.7	213	314	0
LKG 49	54.6	79.4	16.3	211	318	0
EG 105	52.6	77.4	15.9	211	318	0
EG 137	53.2	78.4	16.1	211	320	0
EG 111	53.2	78.3	16.1	211	321	0
Squibb	55.2	81.3	16.7	211	336	0

5–7 days. This agrees with the observations of Winkler & Doty (1961). All the results in Fig. 2 and Table 4 were obtained after the rotations had become constant.

The changes in specific rotation $[\alpha]_D$ with increasing urea concentration are shown in Fig. 2. Below 3M-urea there is very little change in rota-

tion, but as the molarity increases above 4M the rotation increases rapidly and only shows very slight ‘tailing-off’ at 9M. Winkler & Doty (1961) observed that with rabbit γ -globulin there was no ‘tail-off’, even at 10M-urea. The changes observed here for the action of urea on human γ -globulin are very similar to those encountered after the treatment of most proteins with urea, and are taken to indicate unfolding of the molecule with possible $\cdot S \cdot S \cdot$ exchange at high concentrations (Callaghan & Martin, 1962). Table 4 shows the corresponding changes in dispersion constants with increasing urea concentration.

The dispersion constant, λ_c , which is low (213 m μ) in the native protein rises as the urea concentration is increased until in 9M-urea it reaches 224 m μ , a value characteristic of a denatured protein. The intercept, a_0 , of the Moffitt plot follows the rotation in becoming more negative, but b_0 , the slope of this plot, which in the native protein is zero, becomes progressively more negative as the urea concentration is increased above 5M.

This negative shift in b_0 on denaturation is contrary to the results normally obtained at high urea concentrations. The normal interpretation of such a shift is that an increase in right-handed α -helix has occurred, a condition believed to be impossible in urea. A similar increase in negativity of b_0 on denaturation has been observed in a few synthetic polypeptides. For poly-L-alanine, poly-L-leucine (Fasman, 1961) and poly- β -benzyl-L-aspartate (Bradbury, Downie, Elliott & Hanby, 1960; Karl-

Fig. 1. Variation in optical rotation of γ -globulin with pH. Rotation at 589 m μ plotted against pH for γ -globulin soln. (0.5 g./100 ml.) at 20°.

Table 3. Variation of dispersion constant with pH

Measurements made on γ -globulin soln. (0.5 g./100 ml. of aq. 0.15M-buffer) at 20°. Buffer systems: pH 1–3.4, glycine-HCl; pH 3.6–5.6, acetic acid-sodium acetate; pH 8.0–9.5, boric acid-potassium chloride-NaOH; pH 10–12.2, glycine-NaOH; pH 7, unbuffered. $-K$ and λ_c were derived from the Drude equation, a_0 and b_0 from the Moffitt equation.

pH	$-10^{-6} K$	λ_c (m μ)	$-a_0$	$-b_0$
1.1	24.4	223	481	64
2.12	22.3	223	441	59
2.74	20.0	221	401	35
3.12	17.6	218	352	15
3.89	15.6	218	312	15
4.03	15.3	218	303	15
4.64	14.9	217	300	0
5.08	15.3	215	305	0
7.07	15.2	216	305	0
7.78	15.3	215	305	0
8.53	15.3	214	305	0
8.97	15.3	214	305	0
9.52	15.4	214	307	0
10.07	15.5	214	310	0
10.32	15.6	214	314	0
10.73	15.7	214	314	25
12.2	22.3	224	445	59

Fig. 2. Variation in optical rotation of γ -globulin with increasing urea concentration. Rotation at 589 m μ plotted against molar concentration of urea for γ -globulin soln. (0.5 g./100 ml.) at 20°.

son, Norland, Fasman & Blout, 1960), b_0 lies between -100 and -200 for the unfolded molecule.

The increasing negativity of b_0 may arise from the plotting of simple dispersion data in the form of an equation designed to account for complex dispersion; that is, the plotting of data in the form of the Moffitt equation when it may be adequately expressed by the Drude equation. This is permissible only if λ_c calculated from the Drude equation equals λ_0 from the Moffitt equation. In our experiments on denatured γ -globulin, $\lambda_c = 224 \text{ m}\mu$, whereas λ_0 is taken as $212 \text{ m}\mu$. Because of this inequality, b_0 cannot be zero for the random coil.

Spurious b_0 values, the consequence of applying the Moffitt equation, designed to handle complex

dispersion, to materials showing simple dispersion, for which the Drude equation is adequate, have been termed 'pseudocomplexity' by Urnes & Doty (1961).

The solutions of γ -globulin in 2-chloroethanol are affected by the presence of sodium chloride. In concentrations of 2-chloroethanol below 30 % it is necessary to keep the sodium chloride concentration below 0.02 M to prevent precipitation.

The changes in specific rotation at $589 \text{ m}\mu$ with increasing concentrations of 2-chloroethanol are shown in Fig. 3, and the corresponding changes in dispersion data in Table 5. The laevorotation increases as the concentration of 2-chloroethanol rises to 17.5 %, but as the concentration is increased above this value the laevorotation decreases. This sequence has been described for solutions of β -lactoglobulin in dioxan and in dimethylformamide (Tanford *et al.* 1960) and, to a lesser degree, for ovalbumin (Frenkel & Horn, 1960). Although the maximum laevorotation occurs at about 17.5 % 2-chloroethanol, the magnitude depends on the sample.

The dispersion constant λ_c shows an anomalous progression from 5 to 20 % 2-chloroethanol, followed by a steady increase up to 90 % solvent; a_0 (the intercept of the Moffitt equation) follows the changes in rotation, whereas b_0 becomes progressively more negative. The final value reached at 90 % 2-chloroethanol is -233 , slightly less negative than the value quoted by Winkler & Doty (1961). The results obtained for γ -globulin in 20–90 % 2-chloroethanol are of the same form as those obtained for human serum albumin in the same concentrations of solvent. These changes have been interpreted as indicating an increase in helical fold-

Table 4. Variation of dispersion constants with urea concentration

Measurements were made on γ -globulin soln. (0.5 g./100 ml. of aq. urea) at 20° . K and λ_c were derived from the Drude equation; a_0 and b_0 were derived from the Moffitt equation.

Concn. of urea (M)	$-10^{-6}K$	$\lambda_c (\text{m}\mu)$	$-a_0$	$-b_0$
0	15.7	213	314	0
1	15.7	214	314	0
2	15.7	214	318	0
3	16.3	214	329	0
4	16.5	215	336	0
5	19.0	219	383	25
6	23.5	220	472	37
7	28.5	221	570	59
8	30.5	223	610	74
9	32.6	224	656	80

Fig. 3. Change in optical rotation of γ -globulin with increasing 2-chloroethanol concentration. Rotation at $589 \text{ m}\mu$ plotted against percentage of 2-chloroethanol for γ -globulin soln. (0.5 g./100 ml.) at 20° .

Table 5. Variation in dispersion constants with 2-chloroethanol concentration

Measurements were made on γ -globulin soln. (0.5 g./100 ml. of aq. 2-chloroethanol) at 20° . K and λ_c were derived from the Drude equation, a_0 and b_0 from the Moffitt equation.

2-Chloro-ethanol (%)	$-10^{-6}K$	$\lambda_c (\text{m}\mu)$	$-a_0$	$-b_0$
0	15.7	212	314	0
5	16.0	213	320	0
10	17.6	221	356	20
15	22.8	228	454	94
17.5	22.8	231	456	99
20	21.7	233	429	104
30	18.1	242	360	129
40	17.1	244	338	148
50	16.4	251	323	163
60	15.0	262	294	186
70	13.0	264	251	200
75	12.2	269	238	213
80	10.4	276	193	223
90	6.8	290	116	233

ing of the albumin molecule (Callaghan & Martin, 1962). Support for the concept that the increase in laevorotation at low concentration of 2-chloroethanol is a manifestation of molecular unfolding follows from experiments with mixtures of 2-chloroethanol and urea (Table 6).

In 10% 2-chloroethanol containing M-urea the optical rotation of γ -globulin is similar to that in 10% 2-chloroethanol alone. 2-Chloroethanol (10%) and 4M-urea cause an increase in laevorotation to a value slightly greater than at the maximum of the 2-chloroethanol curve. The dispersions are consistent with this increase in laevorotation and indicate slight increase in unfolding over this maximal value. 2-Chloroethanol (40%) and 4M-urea produce, as expected, a smaller increase in laevorotation than in the last sample. The values obtained for both rotation and dispersion correspond to the treatment of γ -globulin with 25% 2-chloroethanol.

To investigate the degree of structural reversibility that follows the various treatments, γ -globulin in selected solvents was dialysed to remove the solvent. In each case dialysis was carried out after treatment for 1 and 7 days. The results of these experiments are shown in Table 7. Dialysis against 0.15M-sodium chloride produces precipitation of the γ -globulin in all cases except after treatment with

2M-urea. Solubility could be maintained by dialysis against distilled water. This fact itself indicates a change in molecular form, since the native protein was insoluble in water but soluble in 0.15M-sodium chloride.

The rotation and dispersion results show that the effect of 2M-urea may be reversed to give rotatory properties similar to those of the native protein. Treatment with 6M-urea for 1 and 7 days and with 9M-urea for 1 day produce samples with increased rotation and dispersion constants, all indicative of some molecular unfolding. Removal of 9M-urea after 7 days gave a solution too turbid for optical measurement.

The reversal of the effect of 2-chloroethanol was also incomplete. Treatment with 20 and 60% solvent for 1 day yielded products with identical optical properties. Treatment for 7 days gave products that showed considerable unfolding over the native γ -globulin but which appear to show the existence of a low helical content. This could, however, be explained by invoking 'pseudocomplexity'.

To test whether the above treatments had destroyed the specific antigenic activity of the molecule, the ability to precipitate specific antisera was examined. Samples treated with 2M-urea, as far as could be judged, showed a normal precipitation

Table 6. *Variation of optical constants with mixtures of 2-chloroethanol and urea solution*

Measurements were made on γ -globulin soln. (0.5 g./100 ml. of aq. mixtures of the two solvents) at 20°. K and λ_c were derived from the Drude equation, a_0 and b_0 from the Moffitt equation.

	$-\alpha_D$	$-\alpha_{500\text{ m}\mu}$	$-10^{-6} K$	λ_c (m μ)	$-a_0$	$-b_0$
10% 2-Chloroethanol + 1M-urea	59.2°	88.1°	17.5	227	348	62
10% 2-Chloroethanol + 4M-urea	78.4	116.9	23.1	229	463	89
40% 2-Chloroethanol + 4M-urea	66.9	100.8	19.3	242	390	121

Table 7. *Dispersion data of γ -globulin after dialysis from urea and 2-chloroethanol*

2M-Urea and 6M-urea were dialysed for 3 days against 0.15M-NaCl. 9M-urea and 20% and 60% 2-chloroethanol were dialysed for 3 days against distilled water. 9M-Urea after 7 days gave a solution too turbid for optical measurement. Protein nitrogen was determined on the protein solutions after dialysis. The constants were derived as stated in the foregoing Tables. All dialyses were at 20°.

Dialysis from	$-\alpha_D$	$-\alpha_{500\text{ m}\mu}$	$-10^{-6} K$	λ_c (m μ)	$-a_0$	$-b_0$
2M-Urea after						
1 day	53.5°	79.0°	16.1	215	325	0
7 days	53.9	79.5	16.2	215	329	0
9M-Urea after						
1 day	64.2	95.0	19.2	219	394	0
7 days			Precipitate			
6M-Urea after						
1 day	56.5	83.4	17.0	215	332	0
7 days	60.8	89.9	18.2	218	356	0
20% 2-Chloroethanol after						
1 day	67.7	100.0	20.3	217	405	20
7 days	70.1	103.9	20.9	221	421	40
60% 2-Chloroethanol after						
1 day	67.7	100.0	20.3	217	405	20
7 days	74.0	109.9	22.0	223	443	54

Fig. 4. Reaction of γ -globulin after dialysis from urea and 2-chloroethanol against specific anti- γ -globulin serum from rabbit. 20 μ l. of antibody serum was used against the equivalent of 10 μ l. of 0.5 g. of untreated γ -globulin/100 ml. ●, Antibody serum. ○, 2M-1 and 2M-7: dialysed from 2M-urea after 1 and 7 days; 9M-1 and 9M-7: dialysed from 9M-urea after 1 and 7 days; 20%-1 and 20%-7: dialysed from 20% 2-chloroethanol after 1 and 7 days; 60%-1 and 60%-7: dialysed from 60% 2-chloroethanol after 1 and 7 days.

reaction. Samples treated with 9M-urea, 20 and 60% 2-chloroethanol gave a much reduced and modified precipitation reaction (Fig. 4).

DISCUSSION

The detailed studies of Porter (1962) suggest that γ -globulin consists of four cross-linked polypeptide chains. Reduction in the presence of urea (Edelman & Poulik, 1961) causes fragmentation through $\cdot S \cdot S \cdot$ cleavage, with the production of light chains (mol.wt. about 20000), the B chains, and heavy chains (mol.wt. greater than 50000), the A chains. The latter constitute 70% of the native molecule. The complete γ -globulin molecule may be visualized as in the representation of Scheme 1.

Stabilization of the molecule would be achieved by the formation of hydrogen bonds, both interchain and intrachain, the gross external archi-

Scheme 1

ture of the molecule in solution depending on the formation of hydrophobic bonds, as the non-polar side chains escape the aqueous environment. The opportunity to form interchain hydrogen bonds is greatest in the 'head' area, where there is close apposition of four polypeptide chains. An area of such interchain hydrogen bonds could resemble the condition known as the β -form, found in certain synthetic polypeptides and postulated in globular proteins. This conformation has characteristic dispersion properties that contribute to the overall optical rotatory dispersion of the protein. Wada, Isuboi & Konishi (1961) have shown that the rotation and the dispersion contributions of the β -form are both positive whereas the other normally encountered conformation, the right-handed α -helix, contributes a positive rotation and negative b_0 value. It seems reasonable to assume that a protein containing both conformations would have dispersion properties quite different from those normally observed, e.g. albumin (Callaghan & Martin, 1962), and it might be expected that changes in environment would affect one structure in preference to the other and so cause abnormal transitions on treatment of the molecule with urea and 2-chloroethanol. Such a situation exists for γ -globulin and we suggest that the optical rotatory dispersion properties of this protein can be explained on the basis of compensating structures within the molecule and not as a solvent-like effect on a non-helical molecule as outlined in the introduction.

Fig. 2 shows that the changes in $[\alpha]_D$ with increasing urea concentration are of the same form as those obtained for many other polypeptides and proteins. These changes have been interpreted as a decrease in helical folding. Throughout this transition it seems probable that urea will rupture both interchain and intrachain hydrogen bonds, causing complete loss of secondary structure, whereas over-

all molecular expansion will take place as a result of the reduction in hydrophobic bonding (Whitney & Tanford, 1962).

Treatment of γ -globulin with 2-chloroethanol (Fig. 3) does give an abnormal transition. This may be explained by the presence of two compensating structures. Low 2-chloroethanol concentrations are accompanied by an increase in the laevorotation and in the negativity of b_0 (Table 5). This is thought to be due to the removal of a structural form contributing positively to both $[\alpha]_D$ and b_0 : the β -form. The dispersion constants are then characteristic of a molecule having a low helical content. The same result may be achieved by treatment with a mixture of urea and 2-chloroethanol (Table 6), which suggests that a hydrogen-bonded conformation is being disrupted during this transition.

Tanford *et al.* (1960) observed similar changes on the treatment of β -lactoglobulin with dioxan and other organic solvents. They do not consider this to represent the breakdown of a specific conformation, but suggest that it is due to a solvent-like effect on the peptide bonds. This reasoning has been questioned by Kushner (1961), who suggests the β -form as a possible explanation of the anomaly.

The removal of the β -form cannot arise solely through the direct rupture of the interchain hydrogen bonds if it be accepted that 2-chloroethanol potentiates formation of hydrogen bonds (Yang & Doty, 1957; Fasman & Blout, 1960; Wada *et al.* 1961). The explanation must lie in the reduction in hydrophobic bonding, as the non-polar side chains become soluble in solvent, and the accompanying expansion of the molecule. Further increase in 2-chloroethanol produces a decrease in laevorotation but a further increase in the negativity of b_0 . This is consistent with an overall increase in the helical folding, although, as is reasonable to assume, some interchain hydrogen-bonding will also occur and be reflected as a small increase in β -form.

Tentative analysis of the data for the 2-chloroethanol transition has been attempted by using an extended form of the Moffitt equation (1) designed to account for the β -form as well as the α -helix, and using the values of a_0^β and b_0^β , characteristic of the β -form, as calculated by Wada *et al.* (1961).

$$[m']_\lambda = \frac{(a_0^\alpha + f_H a_0^\alpha + f_\beta a_0^\beta) \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{(f_H b_0^\alpha + f_\beta b_0^\beta) \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} \quad (1)$$

Estimations of the percentage of α -helix and β -form are shown (Table 8).

These values are speculative and may be over-estimations. Imahori & Momoi (1962) report the α -helical content of γ -globulin in 90% 2-chloroethanol as 53%. If β -form were also present their estimation would necessarily be low. Imahori & Momoi (1962) consider the possibility of β -form in

Table 8. *Estimated percentage of α -helix and β -form in γ -globulin*

Percentages were calculated by using the extended Moffitt equation and the values of a_0^β and b_0^β characteristic of the β -form calculated as +420 and +840 by Wada *et al.* (1961).

Native γ -globulin in	α -Helix (%)	β -Form (%)
0.15M-NaCl	33	30
17.5% 2-Chloroethanol	30	7
90% 2-Chloroethanol	67	24

the molecule but conclude that the evidence of deuterium-hydrogen exchange and infrared spectra against this conformation are not sufficient to rule out the β -structure. Our own limited infrared analyses offer no evidence to refute the suggestions we have put forward, and in the light of the critical article on infrared analyses of proteins by Beer, Sutherland, Tanner & Wood (1958) it would seem that conformational analyses by this method should be viewed with caution.

Winkler & Doty (1961) cite the content of helix-resisting amino acids, particularly proline, as evidence against helix formation in γ -globulin. In view of the findings with 2-chloroethanol (Winkler & Doty, 1961; Imahori & Momoi, 1962; and this present paper) and with detergent (Jirgensons, 1961) this suggestion seems untenable as proline should resist helix formation even in these solvents.

We regard the presence of right-handed α -helix and β -form as possible within the main framework of Porter's suggested structure for γ -globulin. Porter's finding that fraction III of the papain-digested γ -globulin (the tail of the molecule) is crystallizable, and that it presumably has a regular periodic structure, leads us to suggest that the α -helical regions in the native protein lie in this tail region. Optical-dispersion measurements do not, however, show the presence of α -helix in isolated fraction III (Winkler & Doty, 1961).

The increase in helix at high 2-chloroethanol concentrations is presumably a result of an increase in intrachain hydrogen-bonding of the molecule. These bonds are able to form because of the removal of restraints imposed by the hydrophobic forces in native γ -globulin.

SUMMARY

1. Optical rotatory dispersion has been used as a means of studying conformational changes in human γ -globulin under various environmental conditions.

2. The effects of protein concentration, ionic strength, pH, urea and 2-chloroethanol have been described.

3. The dispersion data were abnormal when compared with those of albumin and it was impossible to explain the results on a simple helix-coil transition involving increase or decrease of intramolecular hydrogen bonds.

4. The results are explained on the basis of compensating structures within the molecule, the particular structures suggested being the right-handed α -helix and β -form.

5. The effect of urea is to cause a decrease in both structures. Low concentrations of 2-chloroethanol produce a decrease in β -form by a lessening of hydrophobic forces; high concentrations of 2-chloroethanol cause an increase in α -helix and β -form through increased hydrogen-bonding.

6. The structure of the molecule is discussed in the light of these and earlier findings.

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