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Conformational Variation in a Human Plasma Lipoprotein†

Charles R. Harmison* and Charles E. Frohman

ABSTRACT: A specific and homogeneous α-2 globulin (a lipoprotein) has been repeatedly isolated from plasma of individual human subjects. The α-2 globulin samples are the same in amino acid and lipid composition, in chromatographic elution properties, and in electrophoretic mobility; but they do differ in their effects on a number of intermediary metabolic processes, in their effect on intracellular tryptophan transport, and in their *in vivo* effects on trained rats. In measurements of the optical rotatory dispersion (ORD) and circular dichroism (CD) of these lipoproteins, differences in conformation were found in a given subject from time to time, and between samples from paired subjects studied at the same time. Among the protein samples from patients with schizophrenia, various amounts of α-helical conformation with f_H up to 0.74 were found, with all patients having their protein in this conformation on one or more occasions. Among α-2 globulin samples from healthy control subjects, either the β and/or random-chain conformation was found. The differences in biochemical and biological activities among these α-2 globulin samples seem to be related to the differences in conformation.

In physicochemical studies on samples of an α-2 globulin isolated from individual subjects, measurements of optical rotatory dispersion (ORD) and circular dichroism (CD) were made. Significant differences in conformation were found, and there was evidence for conformational flexibility. The results of these conformation studies for 79 individual samples of the α-2 globulin, prepared from the plasmas of 30 human subjects, are presented in this report.

Several classes of human lipoproteins have been reported to undergo reversible conformation changes *in vitro* as the temperature was altered, with more α-helical conformation at 0–5°, and with increased β conformation at 37° and above (Scanu *et al.*, 1969; Dearborn and Wetlaufer, 1969). There have been reports that other protein molecules with various amounts of α-helical content can be changed reversibly *in vitro* to the random-chain or the β conformation. Among these proteins are serum albumin (Kolthoff *et al.*, 1960), ribonuclease (Anfinsen, 1962; Epstein *et al.*, 1963), and myoglobin and apomyoglobin (Harrison and Blout, 1965). From studies of the transition temperature (T_m) for proteins as the molecular (ionic) environment was varied, von Hippel and Schleich (1969) concluded that most proteins have "marginal conformational stability," and that folded macromolecules were in a dynamic equilibrium with various unfolded forms. From hydrogen-exchange studies under physiological conditions, others have also concluded that folded native molecules were in equilibrium with unfolded molecules (Hvidt and Nielsen, 1966; Rosenberg and Chakravarti, 1968). These conformational changes are reported to be thermodynamically feasible because the net ΔF/mol is small (Nemethy *et al.*, 1963; Lumry and Biltonen, 1969).

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The isolation of these specific α-2 globulins is a part of our program to investigate the biochemistry of schizophrenia. These α-2 globulins occur at the same levels in the plasmas of schizophrenic patients and control subjects; they are identical in amino acid and lipid composition (Frohman *et al.*, 1971), in their electrophoretic mobility (Frohman *et al.*, 1960a), and in having the same elution pattern in column chromatography (Frohman *et al.*, 1960a). The proteins from these two types of donors do differ in their effects on hexose metabolism (Frohman *et al.*, 1960b), on alanine metabolism (Frohman and Gottlieb, 1969), on tryptophan transport into cells (Frohman *et al.*, 1969), and in their effects upon trained rats (Bergen *et al.*, 1962; D. F. Caldwell *et al.*, unpublished data). For many of the individual donors there has been evidence for changes in conformation over a period of time. For pairs of α-2 globulin samples prepared simultaneously from the plasmas of a control subject and a patient, there were marked differences in conformation. These findings for this specific lipoprotein seem to be consistent with the reports in the literature which describe dynamic equilibria between the α-helical conformation and nonhelical conformations.

Materials and Methods

Conformation Studies with ORD and CD. Optical rotatory dispersion (ORD) was measured on a Cary Model 60 spectropolarimeter; circular dichroism (CD) was measured on the same instrument with a 6002 attachment. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard with a 1.27 correction factor since the sample is a lipoprotein. Protein concentrations were determined on each solution as received. The protein and buffer solutions were filtered through a 0.22 μ Millipore filter before being examined in the instrument. Light path length were 5.0–0.1 cm, depending on the volume of protein solution, its concentration, and the wavelength being studied. The samples were held at 26° by the thermostatic controls of the instrument. The sensitivity was 0.04–0.4° rotation for full scale, with adjustments from 0.04° as necessary. Samples were run at least in triplicate for 620–215 mμ; for the short cell runs, 340–195 mμ, it was standard practice to make five to seven runs. From the specific rotation, $[\alpha]$, the reduced mean residual rotation, $[m']$, was determined by

$$[m'] = \frac{3}{n^2 + 2}\frac{M}{100}[\alpha]$$

Here n is the refractive index of the solvent, and was taken from the tables of Fasman (1963); M is the mean residue molecular weight, and for our calculations was taken to be 115. From triplicate CD measurements the mean specific ellipticity was determined; it was corrected for the refractive index of the solvent, and the mean molar ellipticity, $[\theta']$, in (deg cm^2)/dmol was calculated. Reproducibility of measurements was within 5% for ORD from 600 to 220 mμ and for CD from 250 to 215 mμ; it was within 8–10% for ORD below 220 mμ, and for CD below 215 mμ. Most of the ORD runs, as exemplified in Figure 1, have been made with a time constant of 30, and a chart speed of 2.5 mμ/min. With a smaller time constant and a faster chart speed (as in the very early runs), the Cotton effects were just as clearly delineated. The esthetics of the recent charts have been improved with the time constant = 30, and the slow scan speed.

Moffitt plots were prepared from the charts with light path lengths of 5, 2, or 1 cm, from 600 to 300 mμ with λ_0 = 212 and from 300 to 240 mμ, with λ_0 = 212 or 220 mμ; each of these plots was the average of three or more runs. The linear portions of the Moffitt plots were used to determine b_0 (Gotto *et al.*, 1968). For plots of the Cotton effects, the mean $[m']$ values were determined from five to seven runs, with light paths of 2 or 1 mm. The estimates of helical content, f_H,[1] from the ORD measurements were made with the following equations:

$$f_H = \frac{b_0^{obs} - 100°}{-800°}$$

$$f_H = \frac{[m']_{233}^{obs} + 1770°}{-12,830°}$$

$$f_H = \frac{[m']_{198}^{obs} + 2280°}{+67,880°}$$

The values for f_H = 1.0 and for f_H = 0.0 were those proposed by Carver *et al.* (1966). To determine f_H^{208} from the CD charts, the equation was

$$f_H = \frac{[\theta']_{208}^{obs} + 4000°}{-33,000° + 4000°}$$

The values for f_H^{208} were those proposed by Greenfield and Fasman (1969). The mean value of f_H was determined from $[m']_{233}$, $[m']_{198}$, and b_0 for all samples. For the most recent samples, the f_H was also determined from the CD results.

When the ORD of a protein sample had maximal levorotation at 228 mμ, maximal dextrorotation at 205 mμ, and a crossover near 215 mμ, it was concluded that the β conformation was dominant (Greenfield *et al.*, 1967). When the protein was levorotatory between 600 and 200 mμ and had a trough near 205 mμ, it was concluded that the random-chain conformation was dominant (Sarkar and Doty, 1966).

There was the possibility that the conformation results were related to the respective protein concentrations. This possibility was tested for by using the method of least squares to determine the equation for the line in the graph of concentration (μg/ml) *vs.* f_H. The Pearson product–moment correlation was also determined.

Experimental Subjects. All of the subjects that were used for the α-2 globulin samples for this study were drug-free males. The control subjects were very carefully selected to ensure that they matched the patients with schizophrenia, and that they were in good health; they have been employees at Lafayette Clinic. Their age range was 23–55, with a mean age of 34. The patients have been hospitalized for 2–15 years with a diagnosis of schizophrenia; they were drug free for at least 6 months before they were included in these studies; and they were on a protein- and vitamin-supplemented diet which was isocaloric with that of the control subjects (Crandall *et al.*, 1966). Particular efforts were made to have these two groups similar with respect to diet (Gottlieb *et al.*, 1959) and to exercise (Frohman *et al.*, 1963), in order to avoid experimental

[1] Abbreviations used are: f_H = fraction of α-helical conformation; C (protein) from control subject; Me_2Trm, dimethyltryptamine; HDL, high-density lipoprotein; HMP, hexosemonophosphate; LDL_2, low-density lipoprotein, intermediate fraction; L:P, lactate:pyruvate; NEFA, non-esterified fatty acids; S (protein) from patient with schizophrenia.

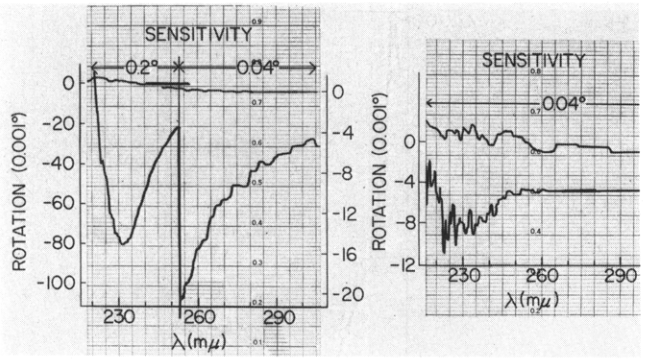

FIGURE 1: Photographs of ORD charts. (a, left) Of an α-2 globulin from a patient. (b, right) Of α-2 globulin from control subject prepared simultaneously. Sensitivities as indicated. For each sample path length = 5.0 cm, protein concentration = 25 μg/ml, solvent was Tris buffer, pH 8.4, 0.004 M.

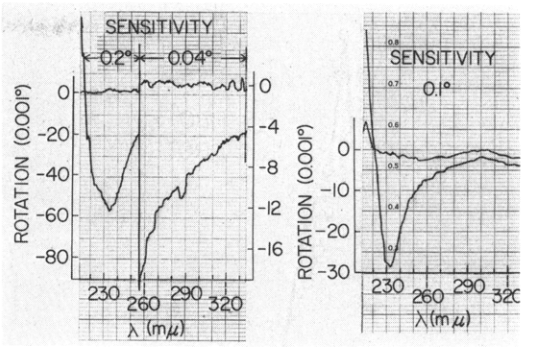

FIGURE 2: Photographs of ORD charts of α-2 globulin samples from patient M. Path length = 2.0 cm; (a, left) concentration = 77 μg/ml; sensitivity as indicated; plasmapheresis on 2-2-70; solvent same as in Figure 1. (b, right) Concentration = 13 μg/ml; plasmapheresis on 8-5-70; other conditions same as for part a. Sample of part a is depicted as third sample for patient M (Figure 9) and sample of part b is the fifth sample for patient M (Figure 9). Plot of Cotton effects for protein in part b is in Figure 6.

artifacts (Kety, 1965). A portion of the patient population was changed periodically with new patients being brought in to replace the patients who were transferred.

Isolation of α-2 Globulin. For all subjects, at least 2 months elapsed between each plasmapheresis. To avoid circadian and other rhythms, each Thursday morning at 8 a.m. to 9 a.m. a patient and a control subject underwent plasmapheresis under fasting conditions, withdrawing one pint of blood from each. The two plasma samples were then fractionated at 4° in parallel and simultaneously. The euglobulins were precipitated by dialysis against five changes of distilled water; the precipitate was dissolved in phosphate buffer (pH 7.4, 0.005 M), chromatographed on DEAE-cellulose columns with a phosphate buffer gradient (0.04 M phosphate with 0.15 M NaCl, pH 4.5, into 0.005 M phosphate pH 7.4), and then subjected to curtain electrophoresis (Beckman Model CP 100) with Tris buffer, pH 8.4 and 0.04 $\Gamma/2$ (Frohman, 1968). The patient's α-2 globulin was identified by assay of [^{14}C]tryptophan accumulation into chicken erythrocytes (Frohman, 1968), by its mobility in curtain (Frohman *et al.*, 1960a), and disc electrophoresis (Frohman *et al.*, 1971). The control protein sample was the one that matched the patient sample in electrophoretic mobility and in elution fraction in column chromatography (Frohman *et al.*, 1960a). With this procedure the end product usually gave a single, narrow band in disc electrophoresis. When protein heterogeneity was found in the disc electrophoresis, the sample was subjected to ultracentrifugation in a sucrose gradient (5–25%) to obtain homogeneity (Frohman *et al.*, 1971). Individual samples varied in final volume (5–23 ml), in concentration (5–130 μg/ml), and in their effect upon tryptophan transport. The average yield was about 100 μg/100 ml of plasma, and ranged from 50 to 150 μg per 100 ml (Frohman *et al.*, 1971). The final products in 0.04 M Tris buffer (pH 8.4) were examined promptly in the spectropolarimeter.

Results

In Figure 1 are photographs of the instrument charts from a pair of α-2 globulin samples that were examined recently in the spectropolarimeter. The protein sample from the patient had a large levorotation at 233 mμ and significant rotation at longer wavelengths (Figure 1a). From $[m']_{233}$ and $[m']_{198}$, f_H = 0.24 and 0.20, respectively; from the slope of the Moffitt plot, f_H = 0.20. The protein sample from the control subject had low optical activity in all regions of the spectrum (Figure 1b), in marked contrast to the patient's protein. This protein sample was levorotatory down through 200 mμ; in the 1-mm cell $[m']_{min} = -3880°$, and was at 205 mμ; these results are compatible with the random-chain conformation being dominant. In the Moffitt plot for this protein $b_0 = +78°$, which is consistent with a nonhelical conformation.

Photographs of charts from two protein samples from patient M are shown in Figure 2 (a and b); the samples were taken 6 months apart. These proteins had considerable levorotation with the trough at 233 mμ. From the ORD parameters for the protein depicted in Figure 2a, the average f_H = 0.28; for the protein depicted in Figure 2b, the average f_H = 0.52.

In Figure 3 in the patterns from two control subjects (Dn and Be) there was much less optical activity, and there were no troughs at 233 mμ. In the 2-mm cell, $[m']_{min}$ was at 205 mμ for both of these protein samples, and there was no dextrorotation. From these ORD results it was concluded that nonhelical conformations were present and dominant. These patterns (Figures 1, 2, and 3) represent the types of patterns found with the proteins from patients and from control subjects.

Plots of the Cotton effects for one of the first pairs of protein samples are shown in Figure 4. From $[m']_{233}$ and $[m']_{198}$ for the protein from the patient (S protein), f_H = 0.72 and 0.74, respectively. The corresponding Cotton effects of the protein (C) from the control subject are consistent with the β conformation being dominant.

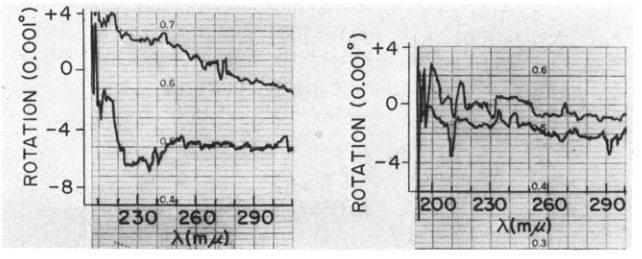

FIGURE 3: (a, left) Photograph of chart of ORD of protein from control subject Be (sample 1 of Figure 10). Path length = 2.0 cm; sensitivity = 0.04°; protein concentration = 15 μg/ml; Tris buffer as before. (b, right) Photograph of chart of protein from control subject Dn (Figure 10). Path length = 2 mm; protein concentration = 21 μg/ml; buffer and other conditions same as for part a.

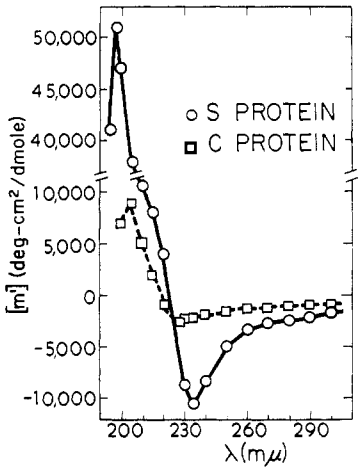

FIGURE 4: Cotton effects for α-2 globulins from patient (S) and from control subject (C). Path length was 2.0 cm for each sample; concentration of S-protein was 4.7 μg/ml, and for C protein was 6.1 μg/ml. Initial concentration of S-protein was 55.5 μg/ml, and initial concentration of C-protein was 41.3 μg/ml; both samples were diluted to fill the 2-cm cell. The patient is Wi, and the control is Br.

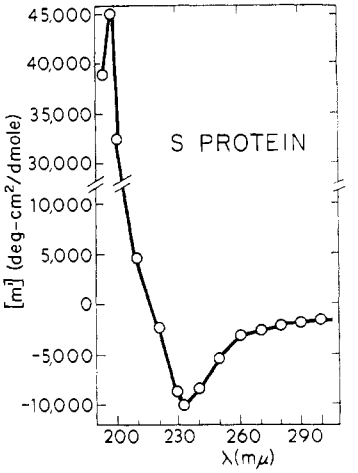

FIGURE 6: Cotton effects for S-protein from patient M, sample 5, of Figure 9. ORD chart of this sample is shown in Figure 2b.

The Moffitt plots for this pair of proteins are shown in Figure 5. From $b_0 = -525°$ for the protein from the patient, $f_H = 0.78$; for the protein from the control subject, $b_0 = +122°$, which is consistent with b_0 values for the β conformation.

In Figure 6 is a plot of the Cotton effects of the S-protein from patient M, whose chart is shown in Figure 2b; strong levorotation is present, with a marked trough at 233 mμ and a significant peak at 198 mμ. From these Cotton effects, $f_H = 0.58$ and 0.51 for 233 and 198 mμ, respectively.

The Cotton effects for protein samples from two control subjects (Da and Dn) are depicted in Figure 7, and are typical of nonhelical conformations, either random chain and/or β conformation. The Cotton effects for these two control protein samples are in marked contrast with the Cotton effects of patient protein samples (Figures 4 and 6).

The CD results from a pair of α-2 globulin samples are shown in Figure 8; the ORD charts for this pair of proteins were shown in Figure 1. From $[\theta']_{208}$ for the helical α-2 globulin, $f_H = 0.24$, which compares very well with the average $f_H = 0.21$ from the ORD results. The CD pattern of the α-2 globulin from the control subject is quite flat and featureless. By the criteria of Greenfield and Fasman (1968) there is no discernible helical conformation in this sample.

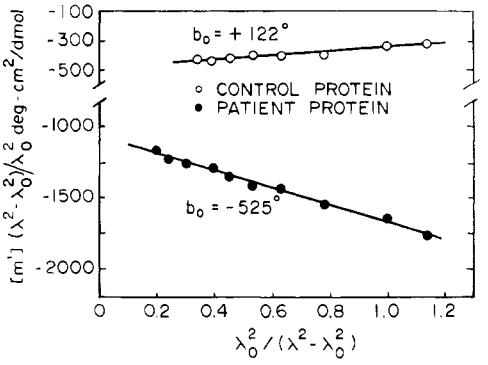

FIGURE 5: Moffitt plots for S- and C-proteins whose Cotton effects are shown in Figure 4.

Forty-eight samples of the α-2 globulin from thirteen patients have now been examined. Each of four patients have provided six samples; other patients have provided fewer samples, down to one sample from each of three patients. In the bar graph of Figure 9 the results of these studies are summarized. Each rectangle depicts the results of one α-2 globulin sample; the f_H or nonhelical conformation is indicated. The results are arranged alphabetically by patient, except for the bottom row, where the results are given for three patients, with one sample apiece. There was good agreement ($\pm 10\%$ or less) among the three or four parameters for determining f_H (*i.e.*, $[m']_{233}$, $[m']_{198}$, b_0, and CD); the average f_H is given in the diagram. Among the protein samples from patients, 35 of the 48 samples had measurable α helix. All 13 of the patients at one time or another had the α-helical conformation in their α-2 globulin samples. The highest $f_H = 0.74$ (patient Wi). For all 48 samples the mean $f_H = 0.17$. With patient F, all six samples were in the α-helical conformation. For patient S, five of six of the S-protein samples had α-helical conformation; for two other patients (M and Lu) four of six samples were α helical. With other patients the incidence of α helix varied, *e.g.*, two of two, two of three, four of five, two of five, and one of one. For seven of the protein samples from the patients the β conformation was dominant, and for six of them the random chain was dominant.

In the bar graph of Figure 10 the results from 31 protein samples from 17 control subjects are depicted. Twenty-six

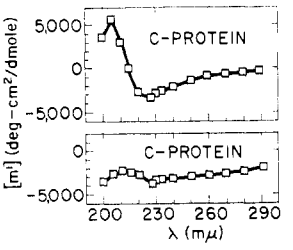

FIGURE 7: Cotton effects for two proteins from control subjects. (a, top) From control Da (sample 2 of Figure 10), concentration = 47 μg/ml. Path length = 2.0 mm and sensitivity = 0.04° for each sample. (b, bottom) From control Dn; in Figure 3b is a photograph of ORD chart for this protein.

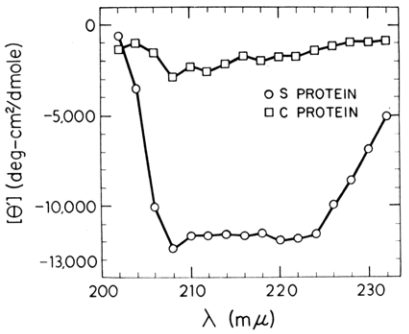

FIGURE 8: Mean residue ellipticity for S- and C-proteins whose ORD charts are shown in Figure 1. Results shown are based on path length = 1.0 cm (to 206 mμ) and = 1.0 mm (below 206 mμ).

of the samples had nonhelical conformation with ten in the β conformation and 16 in the random-chain conformation; the remaining five had small amounts of α helix (f_H = 0.10–0.20). For these 31 protein samples the mean f_H = 0.018. All control subjects but one had nonhelical conformations at one time or another. In 13 of the 17 control subjects, the conformation was always nonhelical, but the conformation did vary in several subjects from time to time. Among the samples with $f_H > 0$, the highest f_H (0.20) was much smaller than the f_H found for many of the patient α-2 globulin samples. The incidence of helical conformation was also much lower for the control subjects than for the patients. Two men were control subjects four times; one was studied three times, and other control subjects were studied once or twice. One control subject whose α-2 globulin was studied only once, had f_H = 0.11.

For the first pairs of samples only the ORD was studied. For the recent samples the CD was studied also. The CD patterns from the patient protein samples usually had troughs at 218–220 and 208 mμ. For the control protein samples the patterns usually had low ellipticity with a single minimum close to 215 mμ (which is typical of the β conformation), or the pattern that is consistent with the random chain conformation being dominant. For a given sample there was good agreement between the ORD and CD as to conformation or f_H.

In all of the tests to establish a dependence of conformation on concentration, the results were negative. When the plot of f_H (ordinate) *vs.* concentration (abscissa) was prepared, and the equation of the line determined by the method of least squares, it was found that: $f_H = 0.258 - 0.00046$ (μg/ml). For this equation the data that were used were from the 35 α-2 globulin samples from the patients, with $f_H > 0$. In the Pearson product–moment correlation, the correlation coefficient, $r = -0.178$, which value signifies no correlation (Fisher and Yates, 1963). The highest f_H (=0.74) was found for the α-2 globulin sample with the lowest concentration (=4.7 μg/ml) (Figure 4). For the matching control sample the concentration = 6.1 μg/ml; this sample was clearly nonhelical. Among the patient α-2 globulin samples with $f_H > 0$, the ten lowest in concentration had a mean concentration = 14.8 μg/ml, and the mean f_H = 0.33. For the nonhelical patient samples, the mean concentration = 21 μg/ml. For the protein samples from the control subjects, the mean concentration = 33 μg/ml. The conformations of these protein samples were clearly independent of concentration.

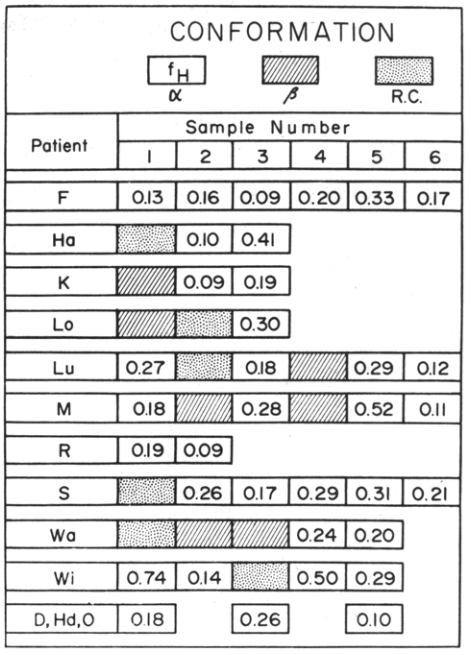

FIGURE 9: Bar graphs of results of conformation studies on 47 α-2 globulin samples from 13 patients. Each rectangle depicts the results on one α-2 globulin sample. Numbers depict average f_H (from b_0, $[m']_{233}$, $[m']_{198}$, and $[\theta']_{208}$ when measured). Shading depicts nonhelical conformations.

Discussion

Similarities and Differences of α-2 Globulin Fractions. The α-2 globulin samples from the control subjects and from the patients are very similar or identical in many ways. These similarities are summarized in Table I. With all of these similarities, it seems that these α-2 globulins are the same protein.

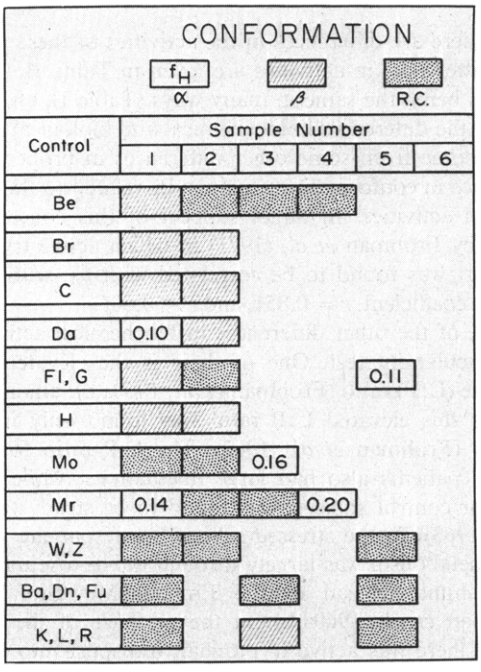

FIGURE 10: Bar graphs of results of conformation studies on 31 α-2 globulin samples from 17 control subjects. Numbers and shadings have same significance as in Figure 9.

TABLE I: Comparisons of Properties of α-2 Globulin Fractions from Control Subjects and from Patients.

Property	From Control Subject	From Patient
Curtain electrophoresis fraction	Tube 18	Tube 18
Column chromatography fraction	31	31
Sucrose gradient fraction	1	1
R_F in disc electrophoresis	0.53	0.53
Lipid moiety: content (%)	80	80
Cholesterol	High	High
Linoleic acid	Present	Present
Oleic acid	Present	Present
Stearic acid	Present	Present
Protein moiety: content (%)	20	20
Lys + Arg (mol %)	14.5	14.8
Asp + Glu	23.2	21.4
His	3.5	3.6
Thr	4.7	4.8
Ser	6.4	6.6
Pro	4.9	5.2
Gly	8.6	8.8
Ala	8.9	9.1
Val	7.3	6.8
Met	Tr	Tr
Ile + Leu	12.3	11.8
Tyr	3.5	3.2
Phe	4.9	4.6
Plasma level (μg/250 ml of plasma)	310 ± 53	293 ± 50
Molecular weight	Near 400,000	Near 400,000

TABLE II: Differences in Activities of Plasma α-2 Globulin Samples from Control Subjects and from Patients.

Activity	From Control Subject	From Patient
Glu transport (μg/ml of erythrocytes per hr)	343	221
Trp transport (dpm × 10^{-3}/g of erythrocytes per hr)	93	142
Serotonin in brain tissue after incubation (ng/g)	8.51	10.07
Me_2Trm in brain tissue after incubation (ng/g)	1.07	1.81
Lactate: pyruvate in chicken erythrocyte incubation	4.9	8.7
Variation in lactate: pyruvate	±10.3	±23.7
Lactate: pyruvate after exercise	13.7	80.0
Insulin effects		
ΔATP synthesis[a] (dpm × 10^{-3}/g per min)	+440	−260
ΔFru → Fru-1,6-P_2 (dpm × 10^{-3}/g per min)	+340	−360
ΔEmbden-Meyerhof pathway (dpm × 10^{-3}/g of tissue)	+0.86	−2.69
ΔHMP pathway (dpm × 10^{-3}/g of tissue)	−6.5	+12.0
Acetate → lactate (dpm × 10^{-3}/mol)	1404	780
NADH + O_2 → NAD^+ + H_2O (% normal)	89.6	86.9
NADPH + O_2 → $NADP^+$ + H_2O (% normal)	94.6	82.0
Rope-climbing time of rats[a] (min-sec)	323	957
Self-stimulation of rats[a] (Δbar presses/min)	+4	−36

[a] These activities were measured *in vivo*. The other activities were from *in vitro* tests.

But there are differences in the activities of these proteins; these differences in activities are given in Table II. With the proteins being the same in many ways (Table I), an explanation for the differences in biochemical and biological activities has to come from some other differences in properties. The difference in conformation seems to be related to these differences in activities. In direct support of this concept is the report by Frohman *et al.* (1971) in which active tryptophan transport was found to be correlated with f_H, with the correlation coefficient, $r = 0.851$, and $P < 0.001$.

Some of the other differences in biochemical activities are of particular interest. One of these is the elevated lactate: pyruvate (L:P) ratio (Frohman *et al.*, 1960a,c); among several diseases this elevated L:P ratio was found only in schizophrenia (Frohman *et al.*, 1962). The L:P ratio for schizophrenic patients also had large fluctuations, while the L:P ratio for control subjects was found to be steady (Frohman *et al.*, 1963). In the stressed schizophrenic patient, carbohydrate metabolism was largely through the hexose monophosphate shunt, instead of the Embden–Meyerhof pathway (Frohman *et al.*, 1960a,b). In the presence of the α-helical protein there was active tryptophan transport into rat brain cells (Warner *et al.*, 1972). The nonhelical protein lacked these activities. There are also two *in vivo* differences in activities of these two forms of the protein. When treated with the protein from a patient's plasma, rats that were trained to climb a rope for a food reward were unable to do so promptly (Bergen *et al.*, 1962). Recently it was found that when electrodes were implanted into rat brains at the "pleasure center" (median forebrain bundle), and the rats were trained to press a bar to receive pleasurable stimuli, they did so incessantly. When the α-helical protein was injected intraventricularly into these rats, they slowed in pressing the bar; with the nonhelical protein, there was no rate change in pressing the bar (D. F. Caldwell *et al.*, unpublished data).

There is some overlap reported in these conformational studies: some patients at times did not have the α-helical conformation, and nine of the control subjects at times had small amounts of α-helical conformation. This latter situation may have occurred because these donors at the time of plasmapheresis were under great stress. For four of these control protein samples, one subject had been up all night (contrary to directions); another had undiagnosed pneumonia at the time, and spent two weeks in bed after his plasmapheresis; and the other two had been subjected to other stresses. None of these four control subjects were included in this report. For the five

control samples which were included in this report, the plasmapheresis was a year or more in the past, and it was impossible to pinpoint lack of sleep, the presence of illness, etc. (These studies on the effects of stress on the α-2 globulin conformation are being continued, but it has not been possible to plan ahead for such studies, in spite of efforts in this direction.) Other plasma parameters have been reported to vary with stress: L:P (Frohman *et al.*, 1966), plasma cholesterol (Wertlake *et al.*, 1958), phosphatidylglycerol (Austin, 1969), and plasma NEFA (Bogdonoff *et al.*, 1959) were all found to be elevated.

The results of these conformation studies were examined by statistical analysis. By the chi-square test, $P < 0.005$; and by the t test, $P < 0.001$. It was therefore concluded that the differences in conformation between the proteins from the patients and the control subjects were real differences.

Conformational Flexibility of Proteins and Lipoproteins. This report on conformational flexibility of a human plasma lipoprotein is consistent with the reports in the literature that both the low- and high-density lipoproteins undergo reversible changes in conformation. With the LDL_2 (d = 1.030–1.042), reversible and "apparently large" thermal conformation changes have been described. At 37° under physiological conditions the α helix, β structure, and perhaps some disordered structure were reported to be present; at 0–5° the α-helical conformation was dominant; and above 37° the β conformation was dominant (Dearborn and Wetlaufer, 1969; Scanu *et al.*, 1969). At room temperature all three conformations were reported to be present (Gotto *et al.*, 1968; Scanu and Hirz, 1968). For the HDL_2 (d = 1.063–1.120), f_H = 0.6–0.7 in phosphate buffer; in 8 M urea or guanidine the α-helical conformation was greatly reduced, but was restored on dialysis. From their thermal studies Davidson and Fasman (1967) proposed the equilibria: α helix ⇄ random chain ⇄ β structure for poly(L-lysine). The temperature studies on the LDL_2 are consistent with the proposal of Davidson and Fasman (1967) that equilibria concentrations among these three conformations can be changed *in vitro*.

Several reports describe the effects of molecular environment and small molecules in altering the conformations of protein molecules. Jirgensons (1966a,b, 1967) reported that in the presence of alkyl sulfates, the conformations of several proteins were altered from essentially nonhelical to partial α-helical conformation. Scanu and Hirz (1968) reported that sodium dodecyl sulfate increased the α-helical content of an LDL_2. In other reports propanol (Jirgensons, 1967) and chloroethanol (Jirgensons, 1966b; Yamagami and Schmid, 1967) increased the α-helical content of several proteins; Yamagami and Schmid found that the change was reversible. The effects of various ions and of ionic strength were discussed by von Hippel and Schleich (1969). These reports on the effects of environment (*e.g.*, urea, alcohols, and surfactants) on protein conformation seem to be relevant to the results reported here. In some reports there is evidence that a small molecule attached to a protein can alter a nonhelical conformation to a partially helical conformation.

When an active patient's α-2 globulin was dialyzed against the control's α-2 globulin, the patient's α-2 globulin lost some activity while the control's α-2 globulin gained considerable activity; in dialysis of the patient's α-2 globulin against saline, there were no losses of activity. From these dialysis experiments Bergen concluded that a small molecule was lost from the patient's α-2 globulin to the control's α-2 globulin; it was not lost in dialysis against saline (Bergen *et al.*, 1962; Bergen, 1965, 1967).

Studies on α-2 Globulin. The lipoprotein described in these studies, with 20% protein and a high cholesterol content (Frohman, 1968) has some properties like the LDL_2 (Granda and Scanu, 1966). With its molecular weight of 400,000 (Frohman, 1968), it is similar to an HDL (d = 1.093) described by Shore (1957). With its high f_H (at times), it resembles the HDL_2 described by Scanu (1965). With its low levels of helix-disrupting amino acids it is very similar to the HDL_2 (Scanu and Hughes, 1962). On a graph of f_H *vs.* per cent of helix-disrupting amino acids (Davies, 1964), the α-2 globulin fell in the same region as myoglobin, β-hemoglobin, and light meromyosin with f_H = 0.75, 0.75, and 0.74, respectively.

The low optical activity that was found for many samples of the α-2 globulin from control subjects is consistent with reports which describe flat ORD patterns and low optical activity for several proteins (Sarkar and Doty, 1966; Yamagami and Schmid, 1967; Dorrington and Tanford, 1968).

The components of plasma are in dynamic equilibria—the proteins, lipoproteins, and hormones. The lipoproteins have a short half-life (Volwiler *et al.*, 1955; Gitlin *et al.*, 1958). With the constantly changing plasma milieu, with the short half-life of lipoproteins and their conformational flexibility, and with conformation being related to molecular environment, it seems to be very plausible that the α-2 globulin can and does change conformation *in vivo*, and that the steady-state conformational equilibria are altered in a given individual at different times.

From the several reports that a small molecule induced α-helical conformation, from the dialysis experiments of Bergen, and with the constantly changing plasma milieu, it is conceivable that there is a dynamic equilibria among the three types of conformation, that the α-2 globulin has measurable f_H *in vivo* in the presence of some small molecule, and in its absence the nonhelical conformations are dominant. The isolation and characterization of this small molecule is of great importance, and several lines of approach are being used. Both in tests with additions of known tryptophan metabolites and in tests with the mass spectrometer, the results to date have been negative. With the yields of the α-2 globulin being in microgram amounts, the concentration level of the small molecule could be in nanograms, or even picograms. The efforts to characterize this small molecule are being pushed very intensively, particularly with mass spectrometry.

Elimination of Experimental Artifacts. A pertinent question is whether the differences in conformation of these two types of α-2 globulin may be an artifact of the isolation procedure. From several lines of evidence this seems most unlikely. The lipid moiety of the LDL_2 contributes to its conformational stability (Scanu *et al.*, 1968; Scanu and Hirz, 1968); and the lipids of the HDL_2 participate in the maintenance of its tertiary structure (Scanu, 1965). When the LDL_2 were succinylated, there were no changes in either the hydrodynamic or optical properties (Scanu *et al.*, 1968). Here at Lafayette Clinic two samples of the α-2 globulin with high f_H were tested for relative conformational stability. The f_H was reduced by heating to 60° in the presence of 8 M urea; neither heat alone nor 8 M urea alone reduced f_H.

In the isolation of the α-2 globulin samples, the dialysis was against distilled water, and the entire procedure was done under mild conditions (see Methods); the two α-2 globulin samples were isolated in parallel and simultaneously (even to the point of having two apparatuses for the curtain electrophoresis). It seems very unlikely that the α-2 globulin samples

from the control subjects would repeatedly lose a small molecule and change conformation, while the α-2 globulin samples from the patients would repeatedly retain a small molecule and their conformations. The final products have consistently manifested differences in conformation. It has been concluded that conformation alteration was minimal during the isolation.

Another question is whether the conformational differences reported here are artifacts arising from differences in diet or exercise between the patient group and the control group; such differences do alter metabolism, and have led to erroneous conclusions in biochemical research (Kety, 1965). Every effort is made to have these two donor groups equivalent in diet (Frohman *et al.*, 1961) and in exercise (Frohman *et al.*, 1963; Frohman, 1968). The plasma parameters of the control subjects were the same, whether they were housed with patients and had the same diet and amounts of exercise (Crandall *et al.*, 1966), or were nonhospitalized Clinic employees (Frohman, 1968). It was concluded that the differences in conformation between control subjects and patients are real, and are not experimental artifacts.

The errors in estimating f_H have been discussed repeatedly (Yang, 1967a,b; Carver *et al.*, 1966; Jirgensons, 1969). To minimize such errors, and to enhance the estimates of f_H, either three or four parameters (b_0, $[m']_{233}$, $[m']_{198}$, and $[\theta']_{208}$) were used. There was good agreement among these values for f_H. The f_H values are given to the second decimal; but rounding to the nearest tenth is more realistic.

From one pint of an individual's blood, the yield of the α-2 globulin was less than 0.2 mg. Thus, the concentrations that were used in this work were only $^1/_{10}$ to $^1/_{100}$ of the recommended concentrations (Jirgensons, 1969). Even though the concentrations were so far from optimal, the differences between the ORD and CD of the patients' protein samples and the controls' protein samples were both marked and highly significant.

Because of the lability of the product (Bergen, 1967; Frohman *et al.*, 1967), samples cannot be stored, frozen, or freeze-dried. Work is in progress to establish $f_H = 1.0$ or 0.0, so that a few samples could be pooled for ultracentrifuge and other relevant measurements.

Apparent Relation of Conformation and Tryptophan Metabolism. Two aspects relative to tryptophan transport merit special comment. One is that, in the presence of the patient α-2 globulin, tryptophan transport was correlated with f_H (Frohman *et al.*, 1971). The other aspect concerns the metabolites of tryptophan. In the brain cells tryptophan can be metabolized through a number of different pathways. One product might be the neurotransmitter, serotonin (Brodie *et al.*, 1966). With excess tryptophan present, excess serotonin may be produced (Fernstrom and Wurtman, 1971), which might alter neurotransmission. Other products might be dimethyltryptamine (Me_2Trm) (Morgan and Mandell, 1969) or other indoles with psychotomimetic actions (Zara, 1956). With excess tryptophan, Me_2Trm or other active indoles could be produced in excess. Thus the helical conformation of the α-2 globulin with its promotion of tryptophan transport, could be related to the brain disturbances as seen in the rope-climbing rats, or the pleasure-seeking rats. On the basis of these results, it is conceivable that it also may be related to some of the mental aberrations that are seen in schizophrenic patients.

From the evidence presented here on the change of conformation of the α-2 globulins, it may be hypothesized that schizophrenia is a type of molecular disease. Phenylketonuria, galactosemia, and sickle cell disease (Nalbandian, 1971) also have been described by this phrase. There are some interesting similarities between sickle cell disease and the observations described in this paper. The sickle cell crisis occurs when the hemoglobin molecule changes conformation reversibly due to the lack of a small molecule (oxygen) (Murayama, 1962). With the α-2 globulin there were reversible changes in conformation; Bergen and others report that a small molecule is involved in the activity of this α-2 globulin. In both diseases there are changes in conformation, and in both diseases a small molecule is either involved or implicated. There is thus the possibility that in patients with schizophrenia, the disease is another example of a molecular disease. This conjecture of course requires a great deal more experimental evidence to be considered a reality.

Scanu *et al.* (1969) speculated that the structural adaptability of the LDL has a physiological significance. In this report a structural adaptability for a human plasma lipoprotein has been described, and this conformational variation has been related to biochemical transport processes. These results may be a partial answer to Scanu's question on the physiological significance of the structural adaptability of a human plasma lipoprotein.

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