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Studies on the Structure of α_1-Acid Glycoprotein. IV. Optical Properties*

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An extensive study of the optical properties of α_1-acid glycoprotein has been carried out. The specific optical rotation was measured over the *p*H range from 1.5 to 13 and its reversibility determined. This property was studied in the presence of several polar organic reagents and in the presence of a wide range of concentrations of NaCl, LiBr, urea, and guanidine hydrochloride. The change in the rotation took place within a limited change in concentration of the latter three reagents. The optical dispersion (3028 to 5893 A) of α_1-acid glycoprotein measured in water, 4 M LiBr, 6 M urea, and 5 M guanidine hydrochloride was plotted according to the single-term Drude and the Moffitt equations. It was concluded that the net helical content of α_1-acid glycoprotein is negligible. The optical rotation of the enzymatically modified glycoprotein (removal of sialic acid and galactose) was determined in water and in the presence of 8 M urea. The oligosaccharide units of this protein do not seem to contribute to the conformation or to modify greatly certain solubility properties of the α_1-acid glycoprotein. It is proposed that the special conformation of the single polypeptide chain of α_1-acid glycoprotein is nonhelical and of an as yet unknown type.

Recent studies of the optical properties of nonconjugated proteins have shown that the helical content and the screw sense of the helix of some of these macromolecules can be calculated on the basis of the Moffitt equation (Symposium, 1960; Proceedings, 1960; Blout, 1960; Urnes and Doty, 1962; Moffitt and Yang, 1956; Urnes *et al.*, 1961; Kendrew *et al.*, 1961). Glycoproteins have been investigated only to a small extent with respect to their optical characteristics. They raise, in addition to the problem of determining the conformation of their polypeptide chains, the question as to what extent their oligosaccharide moieties contribute to the three-dimensional structure and stability of such protein molecules.

α_1-Acid glycoprotein (Schmid, 1953; Weimer *et al.*, 1950), because of the ease with which it can be prepared in a state of extremely high purity and because of its special chemical composition and unique solubility properties, represents an almost ideal protein for the study of these aspects. Prior to this study, Jirgensons (1957, 1958) reported on some optical rotation and dispersion measurements of α_1-acid glycoprotein which we confirmed here. In the present paper further optical properties of this protein are described and answers to some of the above-mentioned problems are presented.

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Material and Methods

α_1-Acid glycoprotein was isolated from pooled normal human plasma by a procedure described earlier (Schmid, 1953). On free moving boundary electrophoresis at *p*H 8.6 it revealed 98 to 100% α_1-globulin.

The isoionic form of α_1-acid glycoprotein was prepared by deionization with the aid of ion-exchange resins (Dintzis, 1952). The sialic acid-free derivative of this glycoprotein was obtained by removal of the sialic acid with neuraminidase. From this protein derivative approximately 75% of the galactose was cleaved off with a bacterial β-galactosidase. The concentration of the enzymatically modified glycoproteins was obtained by dividing the optical density at 278 mμ of the solutions by the E_{278} value of native α_1-acid glycoprotein of 8.93. Thus, the optical rotation refers to the same polypeptide content.

Partial but irreversible denaturation of α_1-acid glycoprotein was effected at *p*H 6 by incubation at 37° in an ethanol-ether-water mixture (1:1:1 v/v) for 3 months. Ultracentrifugation of the resulting preparation showed the following analysis: Three components with sedimentation coefficients of 3.3, 11, and 20 *S* and with relative concentrations of 36, 60, and 4%, respectively. These data indicated that, as judged by the sedimentation behavior, approximately one-third of the protein had not been altered.

For the measurements of the optical rotation a polarimeter (F. Schmidt and Haensch, Berlin) equipped with a Rudolf photoelectric polarimeter attachment, Model 200, and a sodium lamp were employed. All measurements were carried out in 10-cm tubes at 22° to 24°. Eleven readings were taken of each solution and the specific optical rotation was calculated from their average. The obtained $[\alpha]_D$ values were not corrected for refractive index of the solvent. A Rudolf photoelectric spectropolarimeter (high precision) served to measure the optical rotation at wave lengths from 3028 to 5893 A. The reduced mean residue

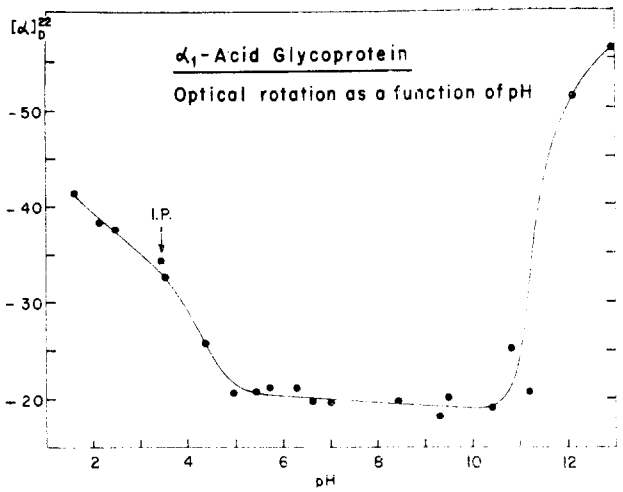

FIG. 1.—The specific optical rotation of α_1-acid glycoprotein measured over a *p*H range from *p*H 2 to 13.

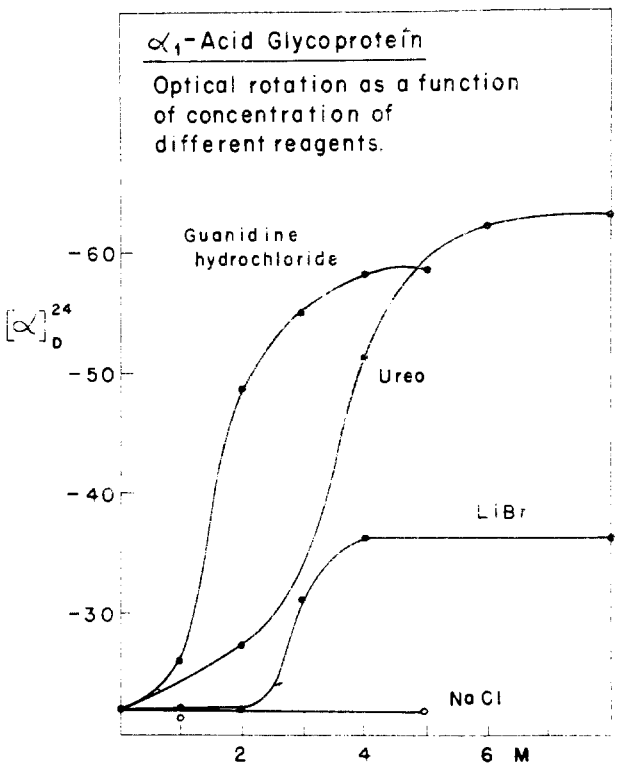

FIG. 2.—The optical rotation of α_1-acid glycoprotein determined at different concentrations of NaCl, LiBr, urea, and guanidine hydrochloride, respectively.

optical rotation was obtained by the following equation: $[m'] = \frac{3}{n^2+2} \cdot \frac{m}{100} [\alpha]$. For m, the average molecular weight of the amino acid residues, a value of 118 was assumed. In the Lorentz correction factor, $\frac{3}{n^2+2}$, n is the refractive index of the respective solvents. The change of the refractive index with wave length was not taken into account. The following n-values were used: 1.395 for 4 M LiBr, 1.383 for 6 M urea, and 1.416 for 5 M guanidine hydrochloride. These values were determined at 24° with a Spencer refractometer made by American Optical Co.

For the determination of the influence of the *p*H upon the optical rotation of α_1-acid glycoprotein, an aqueous solution with a protein concentration between 4 and 5% was prepared. The exact protein content was calculated from the optical density at 278 mμ of a 100-fold diluted aliquot and the extinction coefficient of 8.93 (Schmid, 1953). One-ml aliquots of the stock solution were mixed with increasing amounts (0.01 to 0.1 ml) of 1 N HCl or NaOH and made up to 2 ml with distilled water. After the optical rotations had been determined, the *p*H was measured (Beckman *p*H meter, Model G).

The solutions used for measuring the optical rotation in presence of NaCl, LiBr, guanidine hydrochloride, and urea were prepared by adding to 1 ml of the mentioned stock solution the required amount of solid reagent and distilled water to give the final volume of 2 ml.

Heat-denatured α_1-acid glycoprotein was made by heating aliquots of the same stock solutions (*p*H 5.8) in sealed tubes at 100° for different periods of time.

The solutions for the determination of the optical rotation of the enzymatically modified glycoproteins were prepared by adding to the required amount of lyophilized protein distilled water and, if necessary, solid urea to obtain a volume of 2 ml.

RESULTS

I. Optical Rotation of α_1-Acid Glycoprotein

The specific optical rotations of the native and modified α_1-acid glycoprotein were determined at the D-line only, except those for the optical dispersion studies as described in Section III.

A. pH-Dependence.—The specific optical rotation of α_1-acid glycoprotein was determined within the *p*H region from 1.6 to 13, as shown in Figure 1. From *p*H 5 to 10.5 this property was almost constant, although a slight but definite decrease from −21° to −19° was noted. The rotation increased sharply to −52° in the *p*H range from 11 to 12.5 and to −57° if the *p*H was adjusted to 13. In the acid *p*H region a less drastic change occurred: at *p*H 1.6 the optical rotation rose to only −42°. The isoionic form of this protein, of which the aqueous solution showed a *p*H of 3.4, exhibited a value of −34° which was in agreement with the expected rotation deduced from the curve in Figure 1. After neutralization of this solution, dialysis against water, and lyophilization, the optical rotation at neutrality was found to be −19°. The rotation of the isoionic protein in *p*H 7.4, Γ/2 0.3 phosphate buffer was −21°. However, the protein that was recovered from a solution which had been adjusted to *p*H 1.25 exhibited a specific rotation at neutrality of −32° instead of the expected value of −20°.

The *p*H dependence curve of the specific optical rotation of α_1-acid glycoprotein is grossly comparable to that of many other proteins.

B. Presence of NaCl.—As shown in Figure 2 the presence of NaCl, even in a concentration of 5 M, did not affect the optical rotation of α_1-acid glycoprotein.

C. Influence of LiBr.—In contrast to NaCl, LiBr effected a pronounced change in the optical rotation of α_1-acid glycoprotein (Fig. 2). The change from −21° to −36° occurred within the narrow concentration range from 2.5 to 3.5 M. Interestingly enough, higher LiBr concentrations did not increase the rotation any further. The specific rotation of the protein which had been dissolved in 5 M LiBr was redetermined after removal of this salt and a value of −20° was found.

D. Denaturation by Guanidine Hydrochloride.—The specific optical rotation of α_1-acid glycoprotein was determined as a function of time in the presence of different concentrations of guanidine hydrochloride.

The following observations are of special interest: After an incubation time of 24 hours the same value was obtained as after 10 minutes except in the presence of 2 M guanidine hydrochloride. Under the latter conditions the optical rotation changed from 45° to 48° during the first hour of incubation. The plot of the optical rotation *versus* guanidine hydrochloride concentration (Fig. 2) shows that the largest increment in optical rotation resulted if the molarity of this reagent was increased from 1 to 3 M. No increase in optical rotation was noted at concentrations above 4 M.

The protein solution which contained 2 M guanidine hydrochloride and had been kept at room temperature for 48 hours was dialyzed against water and its optical rotation redetermined. A value of −20° was noted. Ultracentrifugation of the latter protein in 0.1 M NaCl demonstrated a homogeneous component with a sedimentation coefficient of 2.9 *S* at a protein concentration of 1%, corresponding to the expected value (Schmid, 1953).

E. Denaturation by Urea.—The optical rotation of α_1-acid glycoprotein was measured as a function of time of incubation at different urea concentrations. Very short exposure times (10 minutes) led to the final optical rotations at any urea concentration. The major change in the optical rotation was noted in a relatively narrow concentration region, namely, between 2 and 4 M urea (Fig. 2). Ultracentrifugation of an α_1-acid glycoprotein solution containing 4 M urea demonstrated a symmetrical peak. After incubation in 4 M urea for 48 hours the protein was recovered by dialysis against water and subsequent lyophilization. The optical rotation of this preparation was −18°. Ultracentrifugal analysis in 0.1 M NaCl indicated a homogeneous component with a sedimentation coefficient corresponding to that of the untreated glycoprotein.

F. Heat Denaturation at 100° in Distilled Water.—The optical rotation of α_1-acid glycoprotein increased rapidly during the first 4 hours of incubation (Fig. 3). The rate of change decreased gradually, and the maximum rotation of −49° was observed after approximately 24 hours. After 48 hours of incubation the optical rotation had decreased to −44°, probably owing to partial destruction of the protein, as evidenced by the brown color of the solution. Ultracentrifugation of the protein solution which had been incubated for 24 hours showed the following analysis: Four components were observed with sedimentation coefficients of 1.5, 2.8, 5, and 10 *S* and with relative concentrations of 18, 64, 15, and 3%, respectively.

G. Influence of Organic Solvents.—α_1-Acid glycoprotein dissolved readily in 95% hydrazine, 90% phenol, and 90% *m*-cresol. The optical rotations were −42°, −35°, and −18°, respectively. The protein was not sufficiently soluble in chloroethanol to permit analysis. However, in a mixture of equal volumes of chloroethanol and water the rotation could be determined and was found to be −27°. The protein was recovered from the phenol and hydrazine solution and its optical rotation redetermined in water. The values were −19° and −34°, respectively. The latter values seem to indicate that in the presence of hydrazine the protein had been altered to a certain degree (Champagne and Smith, 1960).

II. Optical Rotation of Enzymatically Modified α_1-Acid Glycoprotein

The optical rotation of α_1-acid glycoprotein from which sialic acid had been removed enzymatically was determined in distilled water (*p*H 5.3) and in the presence of 8 M urea. Values of −15° and −63°, respectively, were obtained. These data are compared in Table I with those for the unmodified glycoprotein. Moreover, the specific optical rotation of this enzymatically modified glycoprotein was determined at *p*H 2.48, 3.88, 9.98, 11.09, and 11.48. The obtained values of −32°, −20°, −15°, −14°, −25°, and −42°, respectively,[1] when compared with those of the protein which had not been treated with neuraminidase, would form a curve which is parallel to that shown in Figure 1 displaced by an average of 6°. The differences in optical rotation were 6, 10, 7, 5, 5, and 4°, respectively, at the mentioned *p*H values. Subsequently, the rotation was measured of a preparation from which sialic acid plus approximately 75% of the galactose had been cleaved off. The specific optical rotation in 8 M urea was somewhat less negative than the corresponding values for the native and sialic acid–free glycoprotein. However, the difference in the optical rotation is considered to be not significantly changed.

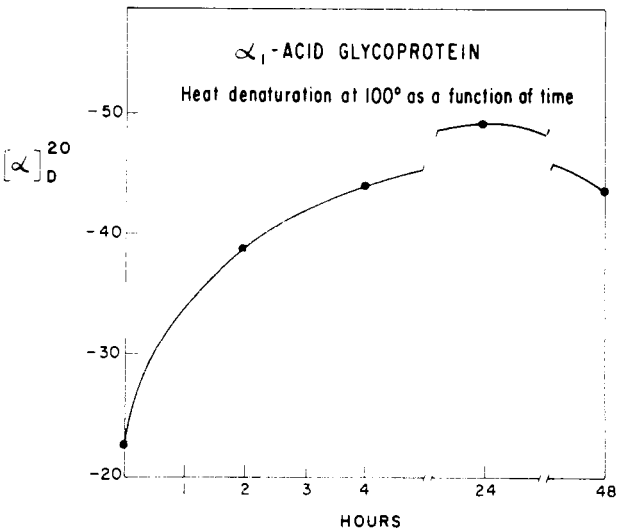

FIG. 3.—Determination of the optical rotation of α_1-acid glycoprotein after incubation at 100° for different periods of time.

TABLE I

THE OPTICAL ROTATION OF α_1-ACID GLYCOPROTEIN

State of the Carbohydrate Moiety	In Water $[\alpha]_D^{20}$	In 8 M Urea $[\alpha]_D^{20}$	$\Delta[\alpha]_D^{20}$
Unmodified	−22°	−64°	42°
Sialic acid removed	−15°	−63°	48°
Sialic acid and 75% galactose removed	−23°	−58°	35°

The two enzymatically modified forms of α_1-acid glycoproteins were also investigated with regard to their solubility properties. They were found to be soluble in the presence of 20% trichloroacetic acid, 20% sulfosalicylic acid, or 10% perchloric acid. Immediate precipitation was obtained by addition of 5% solution of phosphotungstic acid in 2 N HCl. Further, both derivatives remained soluble upon boiling in distilled water. Thus, these properties are identical with those of unmodified α_1-acid glycoprotein (Schmid, 1953).

III. Optical Dispersion of α_1-Acid Glycoprotein

The specific optical rotation was plotted according to the single Drude term equations (Urnes and Doty,

[1] The authors are obliged to Dr. M. Satake for these determinations.

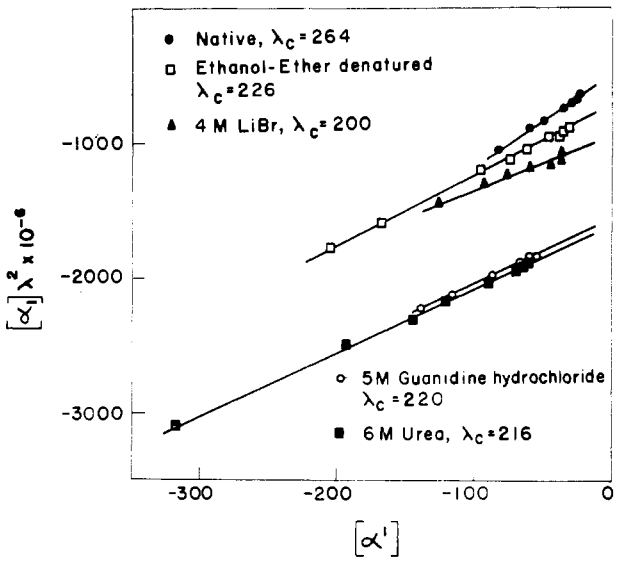

FIG. 4.—Optical dispersion of α_1-acid glycoprotein plotted according to the Drude equation. Measurements were carried out on the native protein dissolved in water (●) and in 4 M LiBr (▲), 5 M guanidine hydrochloride (○), and 6 M urea (■), respectively, and on the ethanol-ether-denatured glycoprotein (□) dissolved in water.

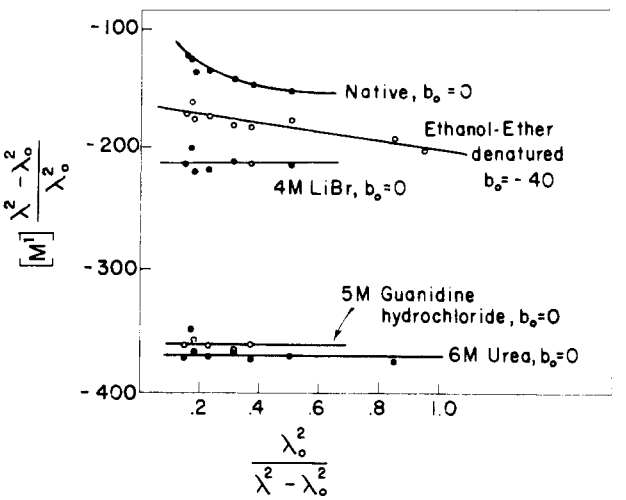

FIG. 5.—Optical rotatory dispersion of α_1-acid glycoprotein plotted according to Moffitt equation with λ_0 = 212 mμ. Measurements were carried out on the native protein dissolved in water, 4 M LiBr, 5 M guanidine hydrochloride, and 6 M urea and on the ethanol-ether-denatured glycoprotein dissolved in water.

1962) (Fig. 4). The data resulted in each case in a straight line. The value of λ_c as computed from the slopes of these lines was found to be 264, 226, 220, 216, and 200 mμ, respectively, for the native and the ethanol-ether denatured protein and for the glycoprotein in the presence of 5 M guanidine hydrochloride, 6 M urea, and 4 M LiBr.

It is of interest to note that the λ_c values of this protein calculated from the results obtained with the 8 M urea and the 5 M guanidine hydrochloride solutions were nearly the same (216, 220 mμ), whereas the corresponding value for the 4 M LiBr solution, being 200 mμ, was much lower. It should be added that the effect of high concentration of the latter reagent on the optical rotation of proteins is still disputed (Bigelow and Geschwind, 1961; Harrington and Schellman, 1958; Mandelkern and Roberts, 1961).

The rotatory dispersion measurements were plotted subsequently according to the Moffitt equation (Proceedings, 1960; Blout, 1960; Urnes and Doty, 1962; Moffitt, 1956); a λ_0 value of 212 mμ was used (Fig. 5). The b_0 values calculated from the obtained straight lines were zero for the protein in 6 M urea, 5 M guanidine hydrochloride, and 4 M LiBr solution. The protein which had been partially denatured by prolonged incubation in a mixture of ethanol-ether-water yielded a b_0 factor of −40. For the native α_1-acid glycoprotein the above-mentioned plot resulted in a curve. A straight line of which the b_0 value was zero was obtained only if these latter data were replotted with a λ_0 value of 220 mμ. No optical rotatory dispersion studies were carried out on the enzymatically modified glycoprotein, because the native and denatured glycoproteins are characterized by b_0 values of zero. Thus, the change in conformation of α_1-acid glycoprotein is not reflected by the Moffitt equation.

TABLE II
OPTICAL CONSTANTS OF α_1-ACID GLYCOPROTEIN

	$[\alpha]_D^{24}$	λ_c (mμ)	b_0 (λ_0 = 212 mμ)
Native (in H_2O, pH 6)	−22°	264	0 (λ_0 = 220 mμ)
Ethanol-denatured, in H_2O	−39°	226	−40
4 M LiBr	−36°	200	0
5 M guanidine HCl	−59°	220	0
6 M urea	−64°	216	0

DISCUSSION

The Moffitt equation is at present considered to be a useful tool for the interpretation of the optical dispersion data of simple proteins in terms of net content and net screw sense of their helices. The b_0 values for α_1-acid glycoprotein calculated from the optical dispersion experiments in 4 M LiBr, 5 M guanidine hydrochloride, or 8 M urea were found to be zero. These findings strongly suggest that in the presence of these two reagents α_1-acid glycoprotein was completely unfolded. The corresponding value of the ethanol-ether-denatured glycoprotein, being −40, indicated perhaps a very small helical content of a maximum of 7%. It is likely, however, that this content is negligible (Winkler and Doty, 1961). Difficulties arose when it was attempted to evaluate the Moffitt plot of the native α_1-acid glycoprotein, because a curve rather than a straight line was obtained (λ_0 = 212 mμ). Thus, the dispersion data were replotted with a λ_0 value of 220 mμ and resulted in a straight line with a b_0 factor of zero, indicating the absence of any helical configuration (in the strictest sense it means the net helical content is zero).

The interpretation of the optical data of α_1-acid glycoprotein can perhaps be brought on firmer ground if a consideration of the amino acid composition is included. The eight proline residues of the glycoprotein molecule, if distributed statistically, would permit the formation of a very limited helical content. In addition, the relatively large amount of serine (8 moles) and threonine (16 moles) and perhaps also the valine (9 moles) residues would probably reduce further the helical content of this protein, as inferred from the behavior of synthetic polymer of these amino acids (Blout *et al.*, 1960). Thus, it is probable that the helical content of the polypeptide chain of native α_1-acid glycoprotein is indeed negligible.

The optical rotation of the enzymatically modified glycoprotein is of special interest. Of particular value is the difference between the rotation observed in water and 8 M urea before and after removal of sialic acid.[2] These differences, being 42° and 48°, could perhaps be interpreted to mean that removal of a relatively large number of negatively charged groupings (16) influences little the secondary structure of this protein. This view appears to be strongly supported by the specific optical rotation of the sialic acid-free protein, which, as a function of *p*H, forms a curve parallel to that of the native protein but displaced by +6°. In addition, after removal of three quarters of the content of galactose, the penultimate sugar of the oligosaccharide units, the mentioned difference did not appear to be significantly changed.

Further, the property of the enzymatically modified α_1-acid glycoprotein of being soluble in the presence of trichloroacetic and perchloric acid and remaining in solution on boiling in distilled water is in contrast to earlier beliefs that these solubility properties are due to either sialic acid or the carbohydrate content. Moreover, it should be added that the denatured form of α_1-acid glycoprotein exhibits the same solubility properties, *i.e.*, denaturation of this protein does not grossly change the classical solubility properties.

Three striking characteristics were found in the present study: (*a*) A large increase in the negative specific optical rotation was noted if α_1-acid glycoprotein was investigated at neutrality in water and in solutions of high urea concentration. (*b*) A negligible net helical content was calculated from the optical dispersion data plotted according to the Moffitt equation. (*c*) The optical rotation of the native protein and of the protein in presence of 8 M urea appears not to be significantly changed by the enzymatic removal of sialic acid. After enzymatic removal of galactose, the corresponding optical rotation remained essentially unchanged. It is of equal importance to note that the curves $[\alpha]_D$ *versus* *p*H of the native and sialic acid–free glycoprotein are parallel to each other. These data seem to indicate that the oligosaccharide units do not contribute significantly to the conformation of this protein. The conformation of α_1-acid glycoprotein, which is of a nonhelical type, is characterized by extremely high stability, as judged for instance by the extremely slow denaturation by heat. This stability must be due to an especially high specificity—an assumption further inferred from the ease with which this protein regains its conformation after incubation with urea, guanidine hydrochloride, and phenol. It is of interest to note that *m*-cresol, in contrast to phenol, does not change the specific optical rotation of α_1-acid glycoprotein, a property shared by other proteins.

[2] Sialic acid is the terminal sugar of the oligosaccharide units of this glycoprotein. The carbohydrate moiety of this plasma protein appears to consist of 5 to 7 branched oligosaccharide units which are linked essentially through the β-carboxyl group of aspartic acid residues to the single polypeptide chain, the backbone of the molecule (Kamiyama and Schmid, 1962b). The oligosaccharide chains appear to be short, branched once or twice, and composed of an average of 14 sugar residues (Kamiyama and Schmid, 1962a).

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