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OPTICAL ROTATORY DISPERSION OF GLOBULAR PROTEINS*

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INTRODUCTION

THE optical rotatory dispersion of globular proteins has been studied recently in several laboratories.[1-9]. This interest is due chiefly to the possibility to correlate the optical rotatory dispersion data with the structure and configuration† of the macromolecules, and good reviews are available on the latter subject.[10-12] The purpose of this paper is to present new results on several proteins, as well as to tabulate the earlier results. A short discussion will be added, indicating the insufficiency of the presently dominating helix-coil transitions model for the treatment of these phenomena.

EXPERIMENTAL METHODS

Most of the 42 proteins mentioned in this paper have been described in previous publications of this and other authors.[1-9,13,14] The hormones prolactin and somatotropin (growth hormone) were gifts from Professor C. H. Li of University of California. The lima bean trypsin inhibitors I and II were isolated in our Laboratory by Dr. T. Ikenaka from a commercial product by chromatographic fractionation, and a detailed report on the inhibitors will be published elsewhere. Ovomucoid was purchased from Pentex, and appeared to be a pure protein containing 9·9 per cent hexose and 15 per cent hexosamine; the protein appeared homogeneous in sedimentation, and it exhibited one slightly asymmetric peak in free boundary electrophoresis. The luteinizing hormone LH_2 was a gift from Dr. D. N. Ward of our Biochemistry Department; a detailed study on the chemistry of this protein will be published by Dr. Ward in due time. The macroglobulins and myeloma globulins were isolated from the blood

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† Dr. E. R. Blout suggests to use the term "conformation" instead of "configuration", if this concerns the long polypeptide chains.

[1] B. Jirgensons, *Arch. Biochem. Biophys.* **78**, 235 (1958).
[2] B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 57 (1958).
[3] B. Jirgensons, *Arch. Biochem. Biophys.* **85**, 532 (1959).
[4] A. Maeda and A. Oikawa, *J. Biochem.* **46**, 495 (1959).
[5] J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg* **30**, 415 (1958).
[6] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[7] B. Jirgensons, *Arch. Biochem. Biophys.* **74**, 70 (1958).
[8] B. Jirgensons, *Arch. Biochem. Biophys.* **85**, 89 (1959).
[9] G. E. Perlmann, *Proc. Natl. Acad. Sci.* **45**, 915 (1959).
[10] C. Schellman and J. A. Schellman, *Compt. Rend. Trav. Lab. Carlsberg* **30**, 463 (1958).
[11] W. Kauzmann, *Ann. Rev. Phys. Chem.* **8**, 413 (1957); *Advanc. Protein Chem.* **14**, 1 (1959).
[12] S. J. Leach, *Rev. Pure Appl. Chem., Austr.* **9**, 33 (1959).
[13] B. Jirgensons and T. Ikenaka, *Makromol. Chem.* **31**, 112 (1959).
[14] W. F. Harrington and M. Sela, *Biochem. Biophys. Acta* **31**, 427 (1959); C. B. Anfinsen in *Symposium on Protein Structure* (Edited by A. Neuberger) p. 223. John Wiley, New York; Methuen, London (1958).

of patients in this and other laboratories; some of the macroglobulins were gifts from Dr. H. F. Deutsch (University of Wisconsin), and more detailed reports on the macroglobulins and myeloma globulins are in preparation.

The proteins were dissolved in water or in aqueous salt solutions or alkalies of low or moderate ionic strength (usually 0·05–0·1). As shown in several previous publications,[1,2] such slight variation of ionic strength does not affect the rotatory properties. (Somewhat exceptional in this respect are some of the redox enzymes, such as aldolase). The pH of the solutions of the native proteins was rather close to the isoelectric point in each case avoiding extreme acid or alkaline reaction. Generally, the native proteins were studied under such conditions when they are maximally stable and yield clear solutions. In some of the denaturation experiments, the solutions were either strongly acid (pH 1·5–2·5) or strongly alkaline (pH 11–12·0), although heating was avoided. Denaturation with guanidine thiocyanate, however, was accomplished by warming the solutions at 50°C for 1–1½ hours. The guanidine thiocyanate was a product of Eastman Coroporation; it was recrystallized from hot methanol. The protein concentration was usually between 0·5–4 per cent, and it was determined either by gravimetric method[1,2] or by micro-Kjeldahl nitrogen analyses,[3] or by both methods for one and the same solution.

The optical rotation was measured with a Rudolph Model 80 photoelectric spectropolarimeter. A Hanovia mercury burner was used as the light source, and the lines at 578, 546·1, 435·8, 404·7, 390·6, 366·3, 365·0, and 334·1 mμ were used in most instances; a sodium lamp, and a zirconium white light source also were used in some experiments.[2] The measurements were made at room temperature of 24–26° ($\pm$2) in 10, 20, or 40 cm semi-micro tubes with glass windows. The latter were fastened with the minimum of stress, and only such windows (end plates) were used that showed a positional variation of the zero point not larger than $\pm$0·02° of the α. The zero points of the tubes, and the instrument without tube, was checked frequently. A quartz control plate certified by the National Bureau of Standards (1614—NBS—1958) also was occasionally used for checking the performance of the instrument.

The dispersion constants were evaluated according to the Yang–Doty method, viz. by plotting $\lambda^2 . [\alpha]$ versus $[\alpha]$. The $[\alpha]_D$ values were calculated from the one-term modified Drude equation. The errors in the dispersion constants λ_0 (or λ_c) were found to be $\pm$2–3 mμ in most cases, while the accuracy in the $[\alpha]_D$ values is in the range of 0·5–2°. This is the reproducibility as obtained with blameless preparations in repeated series of measurements. However, it must be pointed out that the differences in the specific rotation and dispersion constant values found on different preparations of same protein may be much larger than those mentioned. This may be due to either impurities or partial denaturation of the protein. Especially great are the effects produced by colored impurities, e.g. a specimen of impure pancreatic α-amylase[7] yielded a dispersion constant about 30 mμ lower than a colorless pure preparation.[3] Because of the same reason, the results obtained with the not quite colorless preparations of alcohol dehydrogenase and glyceraldehyde phosphate dehydrogenase probably involve larger errors. Less serious sources of error are those associated with defects in the technique of measurement. If the optical activity is low, a considerable error may be caused by the stresses in the tube windows. Other errors may be caused by the weakness and instability of the light source. If a white light source is used, such as zirconium lamp, a considerable error may be caused by the necessity to work with too wide slit.

Results

The results are compiled in Tables 1–4. With respect to the values of the dispersion constants (λ_0 or λ_c), the globular proteins can be classified into three groups (7):

(1) proteins with relatively high dispersion constants (Table 1),

(2) proteins with relatively low dispersion constants (Table 2),

(3) proteins with abnormally low dispersion constants (Table 3).

TABLE 1. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class 1: proteins with *high* λ_0 (λ_0) of 250–280 mμ.

	λ_0	K*	$-[\alpha]_D$	Reference
1. Albumin, egg	266	8·3	30°	1
2. Albumin, serum	267	17·4	63	2,6
3. Albumin, mercapt.	266	18·0	65	2
4. Aldolase	277	7·5	28	3
5. Amylase, bacterial	261	7·2	26	1
6. Amylase, β	277	7·0	26	3
7. Amylase, taka	271	3·3	12	3
	293	3·1	12	4
8. Carboxypeptidase	269	5·7	21	3
9. Dehydrogenase, alcohol†	248	8·7	30	3
10. Dehydrogenase lactic acid	264	11·8	43	3
11. Ficin	261	11·2	40	3
12. α-Glycoprotein, acid	249	6·5	23	2
13. Insulin	267	8·5	31	5
14. Lysozyme	254	13·5	47	6,7
15. Prolactin	276	10·0	37	14*a*
16. Somatotropin	277	7·7	29	14*a*
17. Trypsin inhibitor, lima bean, I	258	7·6	27	14*b*
18. Trypsin inhibitor, lima bean, II	280	4·7	17	14*b*

* If the λ is expressed in mμ, the actual values of K can be obtained by multiplying the K-values of the Tables 1–4 by 10^{-6}.

† According to a recent report of H. Sund [*Biochem. Z.* **333**, 205 (1960)], the λ_c (viz. λ_0 according to the notation used here) of pure and enzymically very active alcohol dehydrogenase is 262 mμ.

It has been found that the high λ_0-values of the proteins of Table 1 decrease upon denaturation (unfolding) considerably. At the same time the specific rotation becomes more negative. The proteins of Tables 2 and 3, on the contrary, show a much smaller change in both λ_0 and $[\alpha]_D$ upon denaturation. The protins of Table 3 either retain their low λ_0-values on denaturation or even show an increase of the constants on unfolding, while the specific rotation becomes more negative. Although the Bence-Jones proteins, γ-globulins, and macroglobulins exhibit a considerable individuality, the dispersion constants of all of them were found in the extremely low range of 180–215 mμ, and they increase on denaturation. The rotatory properties of the denatured globular proteins are compiled in Table 4. Denaturation was ascertained either by the loss of solubility upon removal of the denaturing agent or by the loss of enzymic

[14a] B. Jirgensons, *Arch. Biochem. Biophys.* **91**, 123 (1960).
[14b] B. Jirgensons, T. Ikenaka and V. Gorgurakı, *Makromol. Chem.* **39**, 149 (1960).

TABLE 2. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class II: proteins with *low* λ_0 (λ_c) of 220–245 mμ.

	λ_0	K	$-[\alpha]_D$	Reference
1. Amylase, α, pancreatic	243	13·5	47°	3
2. α-Casein	223	26·9	90	2
3. α-Chymotrypsin	232	18·7	64	3, 5 (p. 455)
4. β-Chymotrypsin	234	18·4	63	7
5. γ-Chymotrypsin	237	18·3	63	7
6. Chymotrypsinogen	235	23·1	79	3, 5 (p. 450)
7. Dehydrogenase, glycerald. phosphate	238	17·3	60	3
8. Globulin, β, metal binding	242	13·3	46	2
9. Lactoglobulin, β	240	9·2	32	2, 5 (p. 396)
10. Ovomucoid	228	21·5	73	14*c*
11. Papain	231	18·5	63	3
12. Ribonuclease	231	21·0	71	5 (p. 458) 6, 7.
13. Trypsin	235	11·7	40	3
14. Trypsinogen	232	13·0	44	3

TABLE 3. ROTATORY DISPERSION AND SPECIFIC ROTATION OF NATIVE GLOBULAR PROTEINS
Class III; proteins with very low λ_0 of 180–220 mμ

	λ_0	K	$-[\alpha]_D$	References
1. Bence-Jones proteins*	180–210	11–17	37–55°	1,2, 8
2. β-Casein	218	42·1	140	2
3. γ-Globulin, normal*	200–217	14–16	44–53	1, 2, and unpublished
4. Luteinizing hormone, LH_2†	212	24·0	80	14*a*
5. Macroglobulin, serum*	196–205	8–10	30–38	14*c*
6. Myeloma globulins, serum*	206–218	11–13	40–45	14*c*
7. Pepsin	216	20·7	69	1, 3, 7, 9
8. Phosvitin‡	207–225	14–24	47–79	2, 7
9. Rennin	210	16·7	55	1
10. Trypsin inhibitor, soy bean	217	23·6	79	3,7, and unpublished

* Variations in the constants of Bence-Jones proteins, γ-Globulins, macroglobulins and myeloma globulins are due to individuality.

† This result was obtained with a 1·0% of sheep luteinizing hormone in acetate buffer of pH 4·7, and ionic strength of 0·05. An even lower λ_0 of 207 was obtained in weakly alkaline borate buffer of pH 8·2.

‡ Variations in the instance of phosvitin are caused by the changes in expansion and unfolding at various pH values.

activity or strong increase of viscosity, usually by two or three criteria, in addition to the changes in the rotatory behavior. 2 M guanidine thiocyanate at 50° appeared to be effective for most proteins, although in some instances 3–4 M guanidine thiocyanate produced a more complete denaturation than 2 M thiocyanate. It was not attempted to include in this Table all results published on this subject by various authors, but only the most definitive and characteristic data are included.

[14c] B. Jirgensons, *Arch. Biochem. Biophys.* **89**, 48 (1960).

The abnormal proteins of group III (Table 3) deserve special attention. Of the well defined crystallized proteins of this class, pepsin has been investigated to a great detail.[1,3,7,9] Five different specimens of one, two or three times recrystallized pepsin possessing full enzymic activity have been examined by the author of this paper. The concentrations of the protein and added neutral salts have been varied; the pH of the solutions was in the range of 4·8–5·5, and autodigestion was avoided by using

TABLE 4. ROTATORY DISPERSION AND SPECIFIC ROTATION OF *denatured* GLOBULAR PROTEINS

Protein	Denaturing agent	λ_0	K	$-[\alpha]_D$	References
1. Albumin, egg	Urea	226	27·5	93°	10
2. Albumin, serum	Urea	221	30·7	103	10
Albumin, serum	Guanidine-HCNS	225	27·0	91	13
Albumin, serum oxidized	Guanidine-HCNS	218	21·3	71	13
3. Aldolase	Alkali, pH 11·2	220	27·5	92	unpublished
4. Amylase, bacterial	Guanidine-HCNS	219	19·2	64	unpublished
5. Amylase, taka	Alkali	213	24·1	80	1
6. Dehydrogenase, lactic acid	Alkali, pH 11·2	225	25·3	95	unpublished
7. Insulin	Urea	226	24·9	84	10
8. Lysozyme	Guanidine-HCNS, 2 M	230	21·2	72	unpublished
Lysozyme	Guanidine-HCNS, 3 M	226	22·2	75	unpublished
Lysozyme	Guanidine-HCNS, 4 M	215	24·9	83	unpublished
9. Prolactin	Guanidine-HCNS, 4 M	207	23·4	77	unpublished
10. Somatotropin	Guanidine-HCNS, 4 M	221	21·3	71	unpublished
11. α-Chymotrypsin	Urea	220	31·6	106	10
α-Chymotrypsin	Guanisine-HCNS, 2 M	218	27·8	93	unpublished
12. Chymotrypsinogen	Urea	224	32·9	111	10
Chymotrypsinogen	Guanidine-HCNS, 2.6 M	209	36·0	119	unpublished
13. β-Lactoglobulin	Urea	225	32·9	111	10
14. Ribonuclease	Urea	220	30·7	103	10, 14
15. Trypsin	Guanidine-HCNS, 2 M	223	26·2	88	unpublished
16. Bence-Jones prot.	Acid or guanid.-HCNS	(218)	(24)		
17. Pepsin	Alkali	224	25·2	85	7
	Alkali, pH 8·9	223	27·5	92	unpublished
1.8 γ-Globulin	Acid or guanid.-HCNS	(220)	(26)	(87)	1, and unpubl.

fresh solutions. All such solutions yielded dispersion constants of 212–216 mμ. Denaturation by means of weak alkali (pH 7·8–9·6) or guanidine thiocyanate always yielded somewhat higher dispersion constants of 222–226 mμ (±2).[1,7] The low dispersion constant for native pepsin has been found also by Perlmann.[9] Also it has been found by Perlmann that the slight rotational changes that occur on long standing of mixtures containing guanidine hydrochloride are due to autodigestion.

The soy bean trypsin inhibitor is another example of proteins of class III that was very thoroughly investigated. Five different preparations of one to five times recrystallized inhibitor were used[1,7]* at various conditions at which the protein retained its inhibiting activity. Altogether 16 series of dispersion measurements have been run with this protein, and, since the solutions were colorless and clear, and the optical activity of the protein is high, the results can be considered as relatively very accurate.

* Unpublished results.

The dispersion constants obtained in these experiments varied within the narrow range of 210–219 mμ. The average value as computed from the best series of measurements (i.e. the one showing the least scatter in the linear plots λ^2. [α] versus [α]) was 217 mμ. The native state was ascertained not only by the enzymic inhibition tests but also by viscosity measurements. The intrinsic viscosity of this protein was found to be 0·050 dl/g at pH 7·8 and ionic strength of 0·05.[7]

The Bence–Jones proteins and the γ-globulins also have been studied to a considerable extent. Although none of them were crystallized preparations, the native state was ascertained by the low specific rotation values and low viscosities of the solutions.

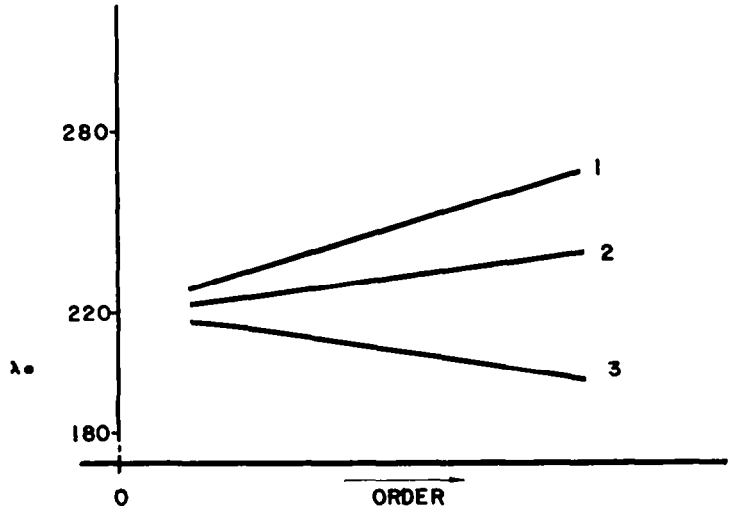

FIG. 1. A qualitative illustration of the dependence of the dispersion constants on the degree of disorganization of the macromolecules of proteins. Curve 1, proteins of group I; curve 2, proteins of group II; curve 3, proteins of group III.

Most of the solutions were quite colorless so that the readings could be extended into ultraviolet up to 365 or even 334 mμ lines of the mercury spectrum. The experimental error, as calculated by the usual statistical methods, in these series of measurements was between 2 and 5 mμ for the dispersion constant.

The changes of the dispersion constants on denaturation (i.e. expansion and disorganization) of the proteins of the three classes are illustrated in Fig. 1. Since a considerable variation is observed in each class, the curves actually should be drawn as bands. Some of the proteins of class III, such as pepsin, show only a slight change of the dispersion constant on denaturation, so that curve 3 shows this change in an exaggerated fashion.

Discussion

Conclusions. The results on the optical rotatory dispersion of native and denatured globular proteins can be summarized in the form of the following three statements:

(1) The one-term Drude rule holds for all colorless native and denatured proteins, when the rotatory dispersion is measured in the wave length range of 365–600 mμ;

(2) The dispersion constants of all *native* globular proteins are within the range of 180–280 mμ;

(3) The dispersion constants of all globular compact proteins converge to a common value of 210–220 mμ on denaturation (expansion, unfolding, disorganization) of the macromolecules.

Rotatory dispersion studies of other authors on polyaminoacids have indicated that the one-term Drude rule holds only for the solutions in which these macromolecules have a random-chain conformation.[6] Since both native and denatured proteins obey the one-term Drude law, one may be tempted to conclude that the macromolecules of native globular proteins do not possess a unique ordered (e.g. α-helical) conformation, but that they are disordered chains. Yang and Doty[6], who thoroughly studied this problem, came to the conclusion that only a relatively small core of the globular macromolecule is orderly folded in aqueous solution, and that this ordered (helical) portion can be increased by using certain non-aqueous solvents. These efforts to reconcile the rotatory dispersion results with a simple helix-coil transition model have been rather successful with several polyamino acids[6,15] but they have been less successful with proteins. For example, this reasoning led to the conclusion that the assumed helical cores must be of one definite screw sense, and that the dispersion constants must decrease on disorganization. In reality, however, several proteins of the class III show an increase of the dispersion constants on disorganization.

Since many of the native globular proteins possessing low dispersion constants have been crystallized (e.g. ribonuclease, pepsin, soy bean trypsin inhibitor), there is every reason to believe that their macromolecules are *ordered*, and that denaturation results in expansion, unfolding, and disorganization of the unique native conformation (or "configuration" in the more common terminology). However, the rotatory dispersion data indicate that these proteins have very little, if any, helical portions, and that their secondary and tertiary structure must differ from that of the proteins possessing high dispersion constants. Aside of the now popular α-helix, other types of secondary structure should be considered in globular proteins. Also the fact should be considered that, due to the interactions of the peptide side groups, the formation of a perfect helix may be hindered. The X-ray structural analysis of myoglobin has shown that the tertiary structure (folding of the chain into a compact body) is very complex;[16] the single polypeptide chain, whatever the secondary structure of it may be, is folded many times. Even if a helical secondary structure would be revealed in the straight sections of the chain, this cannot be the case at the many turns. The variety of the dispersion constants of the native globular proteins can be caused by some variety in the *secondary* as well as *tertiary* structure of the chains in the macromolecules of the proteins. Further studies, e.g. with a very high resolution X-ray diffraction, should show how helical the secondary structure of the polypeptide chain really is, and in what extent the tertiary structure differs in different globular proteins*.

Meantime, however, other attempts can be made in order to correlate the rotatory dispersion data with other properties of the proteins, such as the *amino acid composition* and *primary structure*. It is noteworthy that most of the proteins of class III, such as

* J. C. Kendrew, R. E. Dickerson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips and V. C. Shore [*Nature, Lond.* **185**, 422 (1960)] indeed were able to show that there are helical structures in the macromolecules of crystalline myoglobulin.

[15] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957); M. Idelson and E. R. Blout, *Ibid.* **80**, 4631 (1958); P. Doty in *Proceedings of the Fourth International Congress of Biochemistry* Vol IX. Pergamon Press, London (1959); *Physical Chemistry of High Polymers of Biological Interest* (Edited by O. Kratky) p. 8. Pergamon Press, London, New York (1959).

[16] J. C. Kendrew, *Nature, Lond.* **182**, 764 (1958).

pepsin,[17] γ-globulin, and the Bence–Jones proteins, are rich in the hydroxyamino acids serine and threonine.[3,8] The possibility of interchain hydrogen bonds has already been considered, but covalent ester bonds between the hydroxyl groups and carboxyls of the chains also should be considered.[3,8] It is noteworthy that phosphodiester bonds have already been ascertained in pepsin.[18] In the structural considerations, thus far only the secondary folding of the chain has been considered, and the intrachain hydrogen bonds have been thought to be responsible for the helical folding. However, more and more evidence is accumulating that suggests the importance of other causes for the secondary and tertiary structure, such as electrostatic interaction between charged groups, hydrophoby of the hydrocarbon side groups, as well as the covalent intra- and inter-chain bonds.[11,19] The latter can affect the rotatory properties in two ways: (1) by affecting the asymmetry at the carbon atoms,[20] and (2) by affecting the folding of the chain. The second effect is probably the decisive, although the first should not be altogether neglected. The direct "short range" effects of the side groups, e.g. disulfide bonds, however, are difficult to estimate, since a chemical modification affects not only these groups but also the conformation of the whole chain or at least of some of its parts.[12,13]

The conclusion that the rotatory dispersion of globular proteins is determined by the conformation of the polypeptide chain is supported also by the finding that the dispersion constants of all denatured (unfolded, disorganized) globular proteins converge to the same value of 210–220 mμ. Hence the radicals attached to the asymmetric carbon atoms become unimportant for the numerical value of the dispersion constant (λ_0) when the chain is unfolded and disorganized. While the dispersion constants converge to a common number, the specific rotation of the disorganized proteins seems to depend not only on the degree of disorganization but also on the individual contributions of the asymmetry centers, i.e. the amino acid composition and primary structure of the proteins (see Table 4).

If the differences in the dispersion constants of the native globular proteins would be determined by the ratio of helical to random conformations, all the presumably disordered macromolecules, viz. those with the low λ_0 values, should have higher intrinsic viscosity constants than the presumably helical molecules with their high λ_0. (It is, of course, assumed that in any globular macromolecule the helix is folded thus yielding a relatively symmetrical corpuscular unit). This, however, is not so. The solutions of pepsin, soy bean trypsin inhibitor,[7] as well as those of the Bence–Jones proteins,[21] all possess about the same intrinsic viscosity values as any of the proteins of class I. For instance, the intrinsic viscosity of native pepsin at a moderate ionic strength of 0·01–0·10 is 0·030–0·035,[7,22] while that of serum albumin (class I) is 0·036–0·040 dl/g.[23] Of the proteins compiled in Tables 2 and 3, only the caseins and the macroglobulins have relatively more highly viscous solutions, while the visocity of the phosvitin is strongly dependent on the acidity of the solutions.[2,7]

[17] O. O. Blumenfeld and G. E. Perlmann, *J. Gen. Physiol.* **42**, 553 (1959).
[18] G. E. Perlmann, *J. Gen. Physiol.* **41**, 441 (1958).
[19] C. Tanford in *Symposium on Protein Structure* p. 35. John Wiley, New York; Methuen, London (1958).
[20] J. E. Turner, R. T. Bottle and F. Haurowitz, *J. Amer. Chem. Soc.* **80**, 4117 (1958).
[21] B. Jirgensons, A. J. Landua and J. Awapara, *Biochim. Biophys. Acta* **9**, 625 (1952); B. Jirgensons, *Arch. Biochem. Biophys.* **39**, 261 (1952); **48**, 154 (1954).
[22] H. Edelhoch, *J. Amer. Chem. Soc.* **79**, 6100 (1957).
[23] B. Jirgensons, *Makromol. Chem.* **16**, 192 (1955); C. Tanford and J. G. Buzzell, *J. Phys. Chem.* **60**, 225 (1956).

The results of this paper, moreover, indicate that a low dispersion constant alone is an insufficient characteristic for the state of conformation. In several instances, especially in the group III, the dispersion constants practically do notchange on denaturation, while the solubility, viscosity, specific rotation, and other properties change considerably. This was observed, for example, in the denaturation of γ-globulin.[1,24] Thus a low λ_0 of 220 mμ obviously can be caused by either a certain folded or an unfolded conformation. In the instance of serum albumin, and most other cases (group I), however, the dispersion constant is a safer criterion than the specific rotation.[13]

On the basis of these facts, the following general conclusions can be made:

(1) The wide range of the dispersion constants of the native globular proteins (180–280 mμ) suggests a *variety of ordered compact conformations*. It is suggested that this variety depends, in the first place, on the *amino acid composition* and *primary structure* (residue sequence) of the proteins. While in simple polyamino acids a helix-coil model may sufficiently explain the variations in conformation, in proteins other conformations are indicated by the rotatory dispersion data. The secondary and tertiary structure (conformation) of proteins depends not only on the intra- and inter-chain hydrogen bonds but also on the amount and distribution of the hydrophobic amino acid residues, amount and distribution of electrically charged groups, and on the presence of covalent intra- and inter-chain bonds.[11,12,19,25]

(2) The convergence of the dispersion constants of the various proteins towards a common value of 210–220 mμ on disorganization of the native unique conformation suggests that the *chain conformation* is the dominant factor in determining the dispersion constant, and that a *random-chain conformation* is common to all globular proteins in disorganized state.

Note added in proof: Ch. Tanford, P. K. De and V. G. Taggart have recently published a paper [*J. Amer. Chem. Soc.* **82**, 6028 (1960)] on the rotatory dispersion of β-lactoglobulin in various solvents. These authors arrived at the conclusion that β-lactoglobulin, in spite of the fact that its dispersion constant in aqueous solutions is 240 mμ, must be considered as a non-helical protein. The α-helical conformation in the instance of this protein, according to Tanford *et al.*, is formed only in organic solvents. The absence of the α-helical conformation is explained as being due to the fact that the folding of the chains is caused not by intrachain hydrogen bonding but by the tendency of the lyophobic side chains to escape water. According to Tanford *et al.*, all of the proteins of class III and most of the class II are non-helical in aqueous solutions.

Summary—New and earlier published results on the optical rotatory dispersion of 42 globular proteins are tabulated in this paper. It was found that:

(1) The simple one-term Drude equation holds in all instances of native and denatured proteins. (None of the 42 proteins had absorption bands in the visible spectrum, and the specific rotation was measured in the wave length range of 334–600 mμ).

(2) The dispersion constants of native globular proteins vary within a wide range of 180–280 mμ. Since the dispersion constant is determined by the conformation of the polypeptide chains in the macromolecules of proteins, a variety of unique conformations is indicated in native globular proteins. A low dispersion constant is not necessarily associated with an unfolded and disorganized state.

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(3) The dispersion constants of all denatured (disorganized) proteins approach a common value of 210–220 mμ. This finding is in agreement with the principle that the conformation of the chain determines the dispersion constant, and that denaturation leads to a disordered expanded chain conformation.

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