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Optical Rotatory Dispersion of Some Amino Acids and Criteria of Protein Conformation[1]

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It is shown that the rotatory dispersion of amino acids is well represented by a two-term Drude equation. The parameters of the equation are obtained by least-squares fitting with the aid of an electronic computer, using data for some 42 wave lengths from 650 to 270 mμ. Data are presented for the amino acids alanine, serine, valine, leucine, proline, aspartic acid, glutamic acid, asparagine, ornithine, and lysine, in their several states of protonation and ionization. The root mean square deviation between experimental and computed $[\alpha]_\lambda$ is ±0.21° for the data *in toto*, and 0.09–0.35° for individual solution series. The bearing of the data on problems of helix–random coil transition of proteins and the relation of structure to optical rotation is discussed.

We have shown that precise determinations of the optical rotation of tartaric acid, in solutions of varied composition over a wide wave length span, can be accurately summarized in the form of two-term Drude equations.[2] In addition to thus economically summarizing much data, the variation of the equation parameters with altered environment gave confidence in their use as measures of the relative rotational intensities and peak wave lengths of the implied optical absorptions in the far-ultraviolet.

One of the factors producing a gross change in the rotational behavior was that of ionization. The difference in dispersion curve between, say, a solution of disodium tartrate, and one of tartaric acid in 1 *M* HCl and 0.5 *M* $CaCl_2$, was that between a solution which was positive in its rotation through the spectral range (*ca.* 650–270 mμ) and one which was almost equally negative in its rotation through most of the range. Yet this difference in rotatory dispersion could be related simply to a relatively small change in the Drude parameters.

Rotatory dispersion has been used in studies of protein solutions and has been recommended as a criterion for distinguishing the randomly coiled and helical structured forms of the protein.[3-6] In most of such studies, relatively few wave lengths have been used, and relatively few parameters of solution composition have been controlled. In the light of our experience with the tartaric acid systems, we deemed it important to investigate the spectropolarimetric behavior of the individual amino acids which make up proteins, and then perhaps some of the peptides and proteins, in order to determine whether one could justify the use of rotatory dispersion for conformational analysis of proteins.

This paper summarizes our findings with the optically active amino acids alanine, valine, serine, leucine, proline, aspartic acid, glutamic acid, asparagine, ornithine, and lysine.

Experimental

A detailed description of the experimental techniques has been given in an earlier publication.[2] As in those experiments, in general, rotations were measured at 42 wave lengths in the span 270–650 mμ. Intervals were 10 mμ above 300 mμ, and 5 mμ below this. In view of the low specific rotations of the amino acids, cells of 200-mm. length were frequently used rather than 100-mm. cells. Amino acid concentration was 0.1000 formal unless solubility limits interfered. The amino acids supplied by Nutritional Biochemicals Corp. were used without further purification. Spectroscopic analyses by Miss Doris Cecchi showed less than 0.1% of inorganic impurities. Within the limits set by careful C–H–N analyses performed by Miss Nancy Egan, moisture and possible other impurities were probably less than 1%. An exception was the lysine monohydrochloride which could have had almost 2% moisture. As no definite purity values could be set for the materials, no corrections to the face values of specific rotation were attempted. These would take the form of a proportional constant multiplier for each set of data. Measurements of pH were performed with the Radiometer Model 4 pH meter.

Results

The results of the measurements on alanine, valine, leucine, serine, aspartic acid, asparagine, glutamic acid, ornithine, and lysine are summarized in Table I, in the form of the computed constants for the Drude equation

$$[\alpha]_\lambda = A/(\lambda^2 - \lambda_a^2) - B/(\lambda^2 - \lambda_b^2) \quad (1)$$

Included in the table also are the quantities $(A - B)$, $(\lambda_a^2 - \lambda_b^2) \equiv \Delta$, and $(\lambda_a^2 + \lambda_b^2)/2 \equiv L$. These quantities are significant in view of the least-squares fitting of the data and are discussed in the earlier publication[2] (see also below). The root mean square deviation between the experimental and computed values of $[\alpha]_\lambda$,

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission; reported at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., March 31–April 4, 1963.

(2) L. I. Katzin and E. Gulyas, *J. Phys. Chem.*, **66**, 494 (1962).

(3) C. Cohen, *Nature*, **175**, 129 (1955).

(4) E. R. Blout and M. Idelson, *J. Am. Chem. Soc.*, **78**, 497 (1956).

(5) (a) P. Doty and J. T. Yang, *ibid.*, **78**, 498 (1956); (b) C. Cohen and A. G. Szent-Gyorgi, *ibid.*, **79**, 248 (1957).

(6) P. Doty, *Rev. Mod. Phys.*, **31**, 107 (1959).

TABLE I

SPECIFIC ROTATORY DISPERSION CONSTANTS OF AMINO ACIDS

Species	Solution acidity	Drude equation parameters[a] A	λ_a^2	B	λ_b^2	$(A - B)$	$\lambda_a^2 - \lambda_b^2$	$(\lambda_a^2 + \lambda_b^2)/2$
Alanine·H^+	2 M HCl	298.5195	0.04220239	294.7037	0.04170058	3.816	0.000502	0.04195
Alanine	pH 6.5	360.1463	.04069301	360.1262	.04047647	0.020	.000217	.04058
(Alanine-H^+)	0.25 M NaOH	344.4703	.04989568	343.1793	.04989547	1.291	.000000	.04990
Valine·H^+[f]	2 M HCl	438.4119	.03926320	431.6605	.03866173	6.751	.000602	.03896
Valine	pH 6.06	460.4233	.03465918	459.4160	.03421852	1.0073	.000441	.03444
(Valine-H^+)	0.25 M NaOH	319.3442	.03118233	315.3691	.03050604	3.975	.000676	.03084
Leucine·H^+	2 M HCl	377.4991	.04044465	374.1618	.03980577	3.338	.000639	.04013
Leucine	pH 6.18	274.5246	.03802343	278.2870	.03743202	−3.762	.000591	.03773
(Leucine-H^+)	0.25 M NaOH	314.6331	.03777259	312.5013	.03727019	2.132	.000502	.03752
Serine·H^+	2 M HCl	338.1789	.04230850	334.9272	.04186100	3.252	.000448	.04208
Serine	pH 5.73	318.8132	.03560563	321.5245	.03501944	−2.711	.000586	.03531
(Serine-H^+)	0.25 M NaOH	382.4514	.03479284	383.6311	.03466110	−1.180	.000132	.03473
Aspartic·H^+	2 M HCl	360.9105	.04260890	353.2918	.04253996	7.619	.000069	.04257
Aspartic acid[b]	pH 2.93	446.1446	.03954388	444.9421	.03935718	1.203	.000187	.03945
(Aspartic-H^+)	pH 7.8	374.4645	.03857365	380.6139	.03817383	−6.149	.000400	.03837
(Aspartic-$2H^+$)	pH 12.1	347.8941	.03823479	349.8450	.03755913	−1.951	.000676	.03790
Glutamic·H^+	2 M HCl	361.7220	.03488210	352.7541	.03430429	8.968	.000578	.03459
Glutamic acid[c]	pH 3.25	414.0331	.03695381	410.9175	.03669701	3.116	.000257	.03683
(Glutamic-H^+)	pH 7.94	393.2113	.03567558	395.3116	.03533174	−2.100	.000344	.03550
(Glutamic-$2H^+$)	pH 11.6	336.5308	.03729399	333.6660	.03702243	2.865	.000272	.03716
Asparagine·H^+[e]	pH 1.68	398.0964	.04233022	391.5547	.04225800	7.042	.000072	.04229
Asparagine	pH 5.2	253.8327	.03896011	256.2606	.03865567	−2.428	.000304	.03881
(Asparagine-H^+)[e]	pH 10.42	417.5699	.03106792	421.1466	.03100037	−3.577	.000064	.03103
Lysine·$2H^+$	2 M HCl	1179.298	.04094295	1172.252	.04083325	7.046	.000110	.04089
Lysine·H^+	pH 4.83	441.1781	.03954097	437.5107	.03937528	3.667	.000166	.03946
Lysine	pH 9.93	431.4914	.03917215	427.8984	.03901931	3.593	.000153	.03910
(Lysine-H^+)	0.25 M NaOH	183.0545	.03726084	179.6172	.03679289	3.462	.000468	.03703
Ornithine·$2H^+$[d]	2 M HCl	1350.506	.04207689	1342.576	.04200871	7.930	.000068	.042043
Ornithine·H^+	pH 5.62	432.2491	.03778435	428.066	.03761066	4.183	.000174	.03770
Ornithine	pH 9.80	525.6925	.04052814	522.2509	.04041411	3.442	.000114	.04046
(Ornithine-H^+)[d]	0.25 M NaOH	486.3102	.03752583	482.6644	.03735010	3.646	.000176	.03744

[a] $[\alpha]_\lambda = \frac{A}{\lambda^2 - \lambda_a^2} - \frac{B}{\lambda^2 - \lambda_b^2}$ unless otherwise noted, solutions 0.100 M in amino acid. [b] Amino acid, 0.0375 M. [c] Amino acid, 0.05 M. [d] Amino acid, 0.08 M. [e] Some slow hydrolysis during measurements. [f] Results complicated by indications of possible impurity contributions below 350 mμ.

TABLE II

SPECIFIC ROTATORY DISPERSION CONSTANTS OF PROLINE

Species	Solution acidity	Drude equation parameters[a] A	λ_a^2	B	λ_b^2	$(A - B)$	$\lambda_a^2 - \lambda_b^2$	$\frac{\lambda_a^2 + \lambda_b^2}{2}$	$10A\Delta$
Proline·H^+	2 M HCl	526.0568	0.04120725	542.5659	0.04071333	−16.509	0.000494	0.04096	2.60
		445.3052	.04125130	461.8143	.04066942	−16.509	.000582	.04096	2.64
		306.4864	.04137999	322.9956	.04054139	−16.509	.000839	.04096	2.57
Proline	pH 6.08	403.8729	.04138662	430.2741	.04088043	−26.401	.000506	.04113	2.22
		1282.894	.04116964	1309.298	.04100726	−26.404	.000162	.04109	2.08
		254.6245	.04152665	281.0260	.04073826	−26.402	.000788	.04113	2.01
(Proline-H^+)	0.25 M NaOH	1719.7714	.01997902	1749.4527	.02039450	−29.681	.000415	.02019	..

[a] $[\alpha]_\lambda = \frac{A}{\lambda^2 - \lambda_a^2} - \frac{B}{\lambda^2 - \lambda_b^2}$.

at each of the wave lengths, ranges between ±0.09° and ±0.35° (median ±0.19°) for the individual series. The root mean square deviation for the lumped data is ±0.21°. The actual rotation curves for aspartic acid are plotted in Fig. 1, to illustrate the relations between measured rotatory dispersion and the summarizing parameters in the table. Though ornithine and lysine hydrochloride were weighed, in all cases $[\alpha]_\lambda$ is computed for the molecular formula of the free amino acid.

The parameters for the three proline systems studied are isolated in Table II. One reason is the relatively high deviations between computed and measured rotations (±0.35° to ±0.56°), and the non-Gaussian distribution of these deviations. This suggests the possibility that more than two Drude terms may be needed to completely match the data, as might be the case (for example) if there is an optically active impurity. It may also be that the values of the rotations themselves, all strongly negative (−42° and −81° at 650 mμ for the acid and alkaline solutions, and −258° and −823° at 270 mμ; isoelectric solution intermediate) introduce difficulties in accurate evaluation of parameters. The possible inversion of λ_a^2 and λ_b^2 in the alkaline solution may be noted.

The second reason for isolating the proline material is to illustrate a characteristic of the computations which we have pointed out in connection with the tartaric acid systems.[2] That is, the least-squares computation gives the best values of the Drude parameters of eq. 1 *indirectly* because of the algebraic relation between eq. 1

and eq. 3 (below). The parameters directly optimized are the composite parameters $(A - B)$, L, Δ, and a fourth, $(A\lambda_b^2 - B\lambda_a^2)$, which can be written as a composite of $(A - B)$, L, and the product $(A + B)\Delta$ (*cf.* ref. 2). Because of the wave length distribution of the data and the actual numerical values of the parameters, $(A - B)$ and L are most precisely determined, $(A + B)\Delta$ somewhat less precisely, and Δ probably least precisely. Consequently, the absolute values of A (or B) obtained are dependent on the values for Δ, to which the parameter fitting is least sensitive. In our fitting program, we generally arbitrarily limit the computer to 12 iterations. Unless the arbitrary guess-parameters on which the computation starts are too incongruous, essential fit is achieved in half a dozen iterations. Oscillations then continue which have effect only in the third decimal place of the specific rotations, well outside any relevance (we estimate our experimental error in $[\alpha]_\lambda$ as ± 0.15 to $\pm 0.3°$, depending on molecular weights of solutes). Starting with very different guess-parameters, as was done in the several cases in Table II, 12 iterations on a given set of data may result in differing Δ-values, and corresponding differences in the apparent absolute magnitudes of A and B. The latter are very obvious in casual inspection of Drude parameters; the Δ differences underlying them are less so. Differences in $(A - B)$ and in L are insignificant, and they are a few per cent in $(A + B)\Delta$ (or $A\Delta$).

There exist in the literature a number of papers containing rotatory dispersion data for amino acids. We wish to cite only two, an extensive one[7] based on visual observations and a more recent short report[8] with data extending into the ultraviolet. The Patterson and Brode paper[7] indicates the influence of stage of ionization on the rotations which we also find. In general, it also shows comparable rotations at the wave lengths of measurement. Most significantly, perhaps, the authors recognize from their data, though it is restricted to the wave length span 440–660 mμ, that in essentially all cases representation by two Drude terms is required. Billardon[8] shows graphically the relation of the rotation at fixed wave length to the state of ionization of the entity in solution and the opposed effects of ionizing carboxyl and deprotonating the substituted ammonium group. In addition, Billardon's curves show the anticipated changes in the Cotton (absorption) region to be qualitatively predicted from the changes in rotation seen at longer wave length.

Discussion

One of the first points to attract attention in the data of Table I is the relationship of λ_a^2 and λ_b^2. In the case of tartaric acid[2] it was found that the two critical wave lengths corresponded to absorptions differing by approximately 20 Å. in wave length. The chromophores all contain oxygen, and the wave lengths fell in the region of absorption of the carboxyl group, *ca.* 2116 Å., which was not unreasonable. Since the amino acids have two distinctive chromophores, the carboxyl group and the amino group, it might be expected that the critical wave lengths should correspond to these absorptions, which differ considerably in wave length. On the contrary, the two critical wave lengths are even closer together than in the case of the tartrate

(7) J. W. Patterson and W. R. Brode, *Arch. Biochem.*, **2**, 247 (1943).
(8) M. Billardon, *Compt. rend.*, **251**, 1759 (1960).

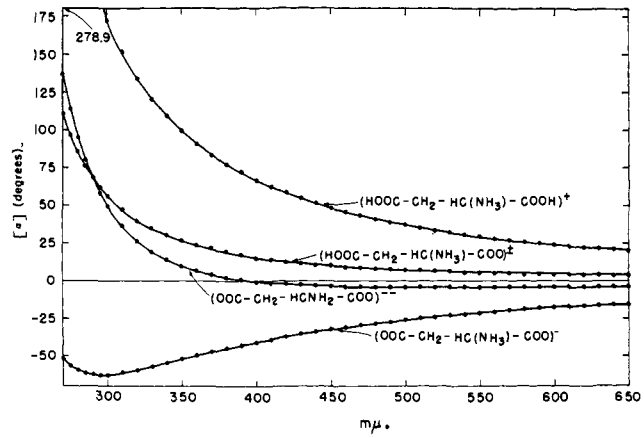

Fig. 1.—Rotatory dispersion of ionization states of aspartic acid.

systems, and in general fall in the region between the two chromophoric absorptions. Only in the case of alanine in strong alkali do the two wave lengths become essentially indistinguishable—that is, the rotation is effectively described by a single Drude term whose numerator or intensity parameter is $(A - B)$, the algebraic sum of the two intensity parameters.

If one considers, together with these facts, that the rotatory dispersion measurements of Woldbye[9] through the absorption region of optically active metal–ion complexes almost uniformly show that there are two Cotton effects very closely spaced, and that the classical circular dichroism and Cotton effect findings on organic compounds[10] and metal ion complexes[11] can also be interpreted in this way, this may be a normal manifestation. That is, in general, rotatory dispersion data will indicate two closely spaced absorptions in the sense of the Drude equation. That this should be the situation is not unreasonable if one considers that one probably always is dealing with interactions between two or more chromophores. Thus, in the classical studies of Kuhn and Braun[10] on solutions of azidopropionic acid methyl ester, a Cotton effect was found in the absorption region for the azide group. However, in substances in which the azide group is accompanied only by the C–H chromophore (*i.e.*, alkyl azides), there is no Cotton effect detected at the azide group absorption.[12] The energy of the C–H absorption is much different from that of the oxygen-containing ester group, and presumably, then, the effective wave length of the interaction is shifted to much lower values.

A second feature of the data is the manner in which the algebraic sum $(A - B)$ relates to structure. Taking the solutions in 2 *M* HCl, the monocarboxylic acids have similar values for this parameter. The values for the dicarboxylic acids resemble each other and differ significantly from those for the monocarboxylic acids. The parameter for the diamino acid is different from that for its monoamino counterpart. All of the acids with linear carbon skeletons resemble each other more than they do proline, with its heteronuclear ring struc-

(9) F. Woldbye, *Acta Chem. Scand.*, **13**, 2137 (1959); O. Kling and F. Woldbye, *ibid.*, **15**, 704 (1961); and private communications.
(10) W. Kuhn and E. Braun, *Z. physik. Chem.*, **B8**, 281 (1930); H. Hudson, M. L. Wolfrom, and T. M. Lowry, *J. Chem. Soc.*, 1179 (1933); T. M. Lowry and D. M. Simpson, *ibid.*, 1156 (1936); J.-P. Mathieu and J. Parrichet, *J. Phys. Radium*, [7] **7**, 138 (1936).
(11) J.-P. Mathieu, Thesis, University of Paris, 1934; *Ann. Phys.*, **11**, 371 (1935).
(12) P. A. Levene and A. Rothen, *J. Chem. Phys.*, **5**, 985 (1937).

ture. These distinctions are perhaps more exact on a molar rotation basis than on the specific rotation one. Thus $M(A - B)$ for alanine, leucine, and serine are approximately 340, 438, and 342; for aspartic and glutamic acids, 1014 and 1319. For dibasic lysine it is 1030, against the 438 for leucine, which lacks the second amino group, and for ornithine it is 1048.

Removal of a proton from a carboxyl group in all cases moves $(A - B)$ to more negative values, and for the dicarboxylic acids the individual alterations for the two carboxyl groups are comparable. Removal of the ammonium proton from the primary amino group gives a slightly smaller change of $(A - B)$, but of the positive sign. The same reaction on proline, with its secondary amino group, pushes $(A - B)$ to more negative values, in contrast. This behavior suggests that to a certain approximation, at least, it may be possible to ascribe a specified numerical influence on the dispersion parameters to a given group in the molecule, or to a given interchange of groups. This can be recognized as a modern version of the relationships originally suggested by Clough[13] and elaborated variously by later workers. This historical relationship has been discussed by Schellman.[14]

The dibasic amino acids lysine and ornithine, after ionization of the carboxyl group, show a characteristic behavior different from that of the other amino acids. The further stages of deprotonation—removal of protons from the substituted ammonium groups—produce quite small changes in rotation and correspondingly small changes in the Drude parameters. Their unique structural feature, to which the rotatory distinctiveness is presumably related, is that following ionization of the carboxyl the molecule has a negatively charged end and a positively charged one. These are able to bend around and approach each other closely, approximating a ring structure. Models also show the two nitrogens to be rather symmetrically disposed on both sides of the carboxylate group. As the α-amino groups in the dibasic acids are weaker bases than the ω-amino groups,[15] the next stage of deprotonation does not affect the electrostatic attraction of the ends of the chain, and the configuration is left intact. The small rotational changes presumably reflect this unchanged geometric arrangement. It is less clear why removal of the next (and last) proton should also produce almost negligible rotational changes. The literature on arginine[7] suggests that it behaves in a similar way. Presumably, for some chain length insufficient to allow ring formation, behavior like that of the monobasic amino acids should be found.

Pursuing structural relations further, the results for serine and alanine show that substitution of a hydroxyl group for a terminal methyl hydrogen has practically no rotatory effect. Substitution of an alkyl chain (leucine) shows relatively trivial effects. However, substitution of a $-COOH$ or $-CH_2COOH$ group (aspartic and glutamic acids) shows a decided effect as does a $-C_nH_{2n}NH_3^+$ group (lysine), with $M(A - B)$ more than doubling. Ionization of the remote carboxyl, similarly, has almost the same effect on the Drude parameters as does ionization of the carboxyl attached to the asymmetric carbon. These relations suggest that perhaps more important, in at least some cases, than vicinal effects in terms of the proximity of a chemical change to the asymmetric carbon are interaction possibilities between energy levels of chromophores.

The amino acids used are all of the levo configuration. Natural tartaric acid is of the dextro configuration.[16] If the Drude terms of the tartaric acid system, A and B, are summed algebraically, as has been done for the amino acids, they go from $+5.67$ to $+13.65$, or a change of $+8$, in going from the fully associated form in acid solution to the fully ionized form in alkaline solution. Two carboxyls are involved. The corresponding change for dicarboxylic L-aspartic acid is about -14. This suggests that comparison of Drude parameters of neutral and ionized forms of optically active carboxylic acids, and perhaps also of the basic and salt forms of amino compounds, may give information on absolute configurations more quickly and easily than crystallographic investigation[16] or synthetic relations with compounds of known (or assumed) configuration. This again may be considered a refinement of criteria suggested by much earlier workers.

Though our measurements are not directly on polypeptides, they do furnish information relevant to current practices in computing degree of helix–random coil transformation in polypeptides (*cf.* ref. 6, for example). Such computations are based on the concept that helical structure *per se* gives a significant optical rotatory contribution,[3] described by the theoretically derived equation[17]

$$[\alpha]_\lambda = \frac{\bar{A}\lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{\bar{B}\lambda_0^4}{(\lambda^2 - \lambda_0^2)^2} = \frac{\alpha}{\lambda^2 - \lambda_0^2} + \frac{\beta\lambda_0^2}{(\lambda^2 - \lambda_0^2)^2} \quad (2)$$

Polypeptides "lacking the helical structure" are those giving an apparent single Drude term; *i.e.*, $\bar{B} = 0$. From this viewpoint, the second term becomes a measure of helix content. If, however,[2] one rewrites the two-term Drude equation (our eq. 1)

$$[\alpha]_\lambda = \frac{(A - B)\lambda^2 - (A\lambda_b^2 - B\lambda_a^2)}{(\lambda^2 - \lambda_a^2)(\lambda^2 - \lambda_b^2)} \quad (3)$$

then in the approximations $(\lambda_a^2 + \lambda_b^2)/2 = L \approx \lambda_a^2 \approx \lambda_b^2$, and $\lambda^2 >> L$, we have

$$[\alpha]_\lambda = \frac{\alpha}{\lambda^2 - L} + \frac{\beta L}{(\lambda^2 - L)^2} \quad (4)$$

The Moffitt equation is therefore effectively a three-parameter approximation to a four-parameter Drude equation. The Moffitt parameter $\bar{B}$ thus depends in apparent magnitude and sign on the relative values of A and B, and of λ_a^2 and λ_b^2 also.

With α-polypeptides, as compared to the free amino acids, the "inversion" of λ_a^2 and λ_b^2 (negative Δ) seems to be characteristic. That is, for both the "helical" and "random coil" forms the rotations assume large

(13) G. W. Clough, *J. Chem. Soc.*, **113**, 526 (1918).

(14) J. A. Schellman, "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(15) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 84.

(16) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature*, **168**, 271 (1951); A. F. Peerdeman, A. J. van Bommel, and J. M. Bijvoet, *Proc. Acad. Amsterdam Ser. B*, **54**, 16 (1951).

(17) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).

negative values at short wave lengths,[18] where the free amino acids generally assume large positive rotation values. With poly-1-benzyl histidine this inversion does not seem to take place[19] or is masked by other factors. If one takes the rotation values for poly-γ-benzyl L-glutamate "practically all in the helix configuration" in dioxane (material used to propagate helical polymerization) from Doty and Lundberg[20] (12 wave lengths from 350 to 750 mμ) and computes Drude equation parameters by our program, one obtains

$$[\alpha]_\lambda = \frac{653.8996}{\lambda^2 - 0.04505702} - \frac{651.5985}{\lambda^2 - 0.04540790}$$

which fits the data within the stated experimental errors. It is seen that the parameters are completely comparable to those for the free amino acid systems in our data, with the exception of the sign of Δ. The $\bar{B}$-value calculated for this polypeptide from our Drude parameters is −166 in the dioxane solution to which the data refer. Corresponding numerical data for polypeptide at higher pH, allegedly following the one-term Drude relation, are not at hand. It will be noted that the L-value of 0.04523 μ^2 (about 2126 Å.) corresponds to the approximate λ_0 usually taken for both "helical" and "random coil" polypeptides. The $(A - B)$ value of about 2.3 accounts for the positive rotations at long wave lengths. If this were the free amino acid, an increase in pH would move this parameter in the negative direction, possibly 5–6 units if it were glutamic acid. Such a change, with the inverted critical wave lengths of the polypeptide, could give the illusion of a one-term Drude description for the dispersion, unless sufficiently careful measurements were made over a sufficiently wide wave length span, and least-squares analyses were performed of the sort in our computer program. The Doty and Lundberg data for longer peptide units, obtained by using the above material as initiator with more L-monomer, are fitted (slightly less well) by the equation

$$[\alpha]_\lambda = \frac{457.0749}{\lambda^2 - 0.045716} - \frac{451.6963}{\lambda^2 - 0.0472547}$$

for which $\bar{B}$ is −4.39. Note that $(A - B)$ and $(\lambda_b^2 - \lambda_a^2)$ seem to have increased, in consequence of the increased chain length, to give the larger $\bar{B}$. The new L-value corresponds to about 2155 Å., still in the conventional range.

We may summarize our position on the rotatory dispersion and conformation relations of the polypeptides as follows.

The amino acids of which the peptides are constituted have rotatory dispersions which are very well described by a two-term Drude equation in which the critical wave lengths ("dispersion parameters") are quite close together, and in which the relative values of the two intensity parameters ("rotatory parameters") play an extremely significant role. These parameters are sensitive to ionization changes and to varied environmental alterations, which give large changes in the rotatory dispersion. For many of these systems the wave length parameters tend toward the absorption region of the carboxyl group in its various states of modification (2000–2200 Å.). The outstanding difference between the rotatory dispersions of the simple amino acids and of the long-chain polypeptides seems to be that, in consequence of the formation of a long sequence of α-peptide bonds, the Drude term of the negative sign, which is associated with the *shorter* wave length dispersion parameter in the monomeric acids, has "crossed over" the positive term and is now at relatively *longer* wave length. This small change results in an apparently grossly different dispersion curve.

We have shown that the Moffitt equation, which has been used widely to distinguish helical from randomly-coiled polypeptide, is a three-parameter, approximate form of the four-parameter Drude equation of two terms, and that the Moffitt parameters may be derived from the Drude parameters. Further, the Drude parameter changes accompanying the alteration from "helical" to "randomly coiled" polypeptide are in magnitude and nature not distinguishable from the corresponding changes that the monomeric amino acids show in response to ionization changes, for example. It therefore seems extremely likely that the rotational changes observed for peptides, with alteration of medium, pH, etc., are related to helix–random coil transformation only insofar as they reflect, at the local unit level, the changes in hydrogen bond relations, geometric strain, solvent interaction, etc., which may, respectively, be responsible for or accompany the overall conformation change. The Cotton effects observed when the 2000–2200 Å. region is studied directly are then simply the expected accompaniment of the dispersion at longer wave length expressed in the Drude parameters.

Compilations of the Moffitt parameters for polypeptides[21] show that the b_0 parameter in "random-coil" (acidic or strongly donating) solvents, for a group of polypeptides having the "characteristic" values of b_0 in "helical" solvents, *ca.* −650, may be not only the expected null, but positive (+50), or even −390, not too different from those in the "helical" solvents. Other polypeptides, in the "helical" solvents, may show null values which do not change in the "random-coil" solvents, or may show large positive values which are hardly altered in the "random-coil" solvent (+540 and +450). At least one has a +80 value in "helical" solvent and increases to +350 in the "random-coil" solvent. Such behavior does not seem to fit as closely as should be expected to the fixed relations assumed[3-6,18,20] between helical structure and optical rotation. If one considers the dispersion behavior, rather, to be largely an expression of the responses of the monomeric constituents to the changes in their acid–base relations with the environment, there is ready explanation in terms of changes measured in the Drude parameters, as indicated above. In this connection also it hardly seems necessary to remind readers of the very considerable effects of solvent on the dispersion curves of even simple molecules, known from early work (*e.g.*, Tschugaeff and Ogorodnikoff[22]).

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