*AN ANALYSIS OF THE OPTICAL ROTATORY DISPERSION OF POLYPEPTIDES AND PROTEINS, III**

By E. Shechter, J. P. Carver, and E. R. Blout

DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL

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In two previous communications[1, 2] it has been shown that the visible and near-ultraviolet optical rotatory dispersion of polypeptides and proteins which are part α-helical, part random can be fitted by a *modified two-term Drude equation:*[2a]

$$[R'] = \frac{A_{(\alpha,\rho)(193)}\lambda^2_{193}}{\lambda^2 - \lambda^2_{193}} + \frac{A_{(\alpha,\rho)225}\lambda^2_{225}}{\lambda^2 - \lambda^2_{225}}. \quad (1)$$

In addition it was found that for a given α-helical content the principal factor which seems to influence the values of $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$ is the dielectric constant of the solvent. This variation is sufficiently small that solvents may be grouped into two categories: those of high dielectric constant ($D > 30$) and those of low dielectric constant ($D < 30$). In each class of solvents a linear relation exists between $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$:

$$A_{(\alpha,\rho)225} = -0.55\, A_{(\alpha,\rho)(193)} - 430 \quad (2)$$

for D greater than approximately 30, and

$$A_{(\alpha,\rho)225} = -0.55\, A_{(\alpha,\rho)(193)} - 280 \quad (3)$$

for D smaller than 30. This linearity was interpreted as meaning that each of the two independent factors, $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$, is a linear function of helix content.

In this communication we will consider the advantages of the modified two-term Drude equation over the methods previously employed for determining helix content, namely, the rotation at the sodium D line, the one-term Drude equation, and the Moffitt equation.

Comparison of the Modified Two-Term Drude Analysis with Previous Types of Analysis.—Comparison of the use of equation (1) and the rotation at the sodium D line: One of the earliest methods of estimating helix content in polypeptides and proteins was to use the rotation at the sodium D line ($[R']_D$).[3, 4] It was assumed that the helix content was linearly related to $[R']_D$. Values of $-100°$ and $0°$ were used for random and fully α-helical polypeptides, respectively, and the helix content was derived by direct interpolation.

From equation (1) we see that $[R']_D$ is given by

$$[R']_D = 0.120\, A_{(\alpha,\rho)(193)} + 0.171\, A_{(\alpha,\rho)225}. \quad (4)$$

Since $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$ are, in general, large and approximately equal numbers with opposite signs, small per cent variations of these parameters will give rise to large per cent variation in $[R']_D$. As an example, we have calculated the values of $[R']_D$ as a function of helix content in solvents of high and low dielectric constant (see Fig. 1). It can be seen immediately that the difference in rotation at the sodium D line is very large depending on whether measurements are made in solvents of high or low dielectric constant; or conversely, a given $[R']_D$ could be

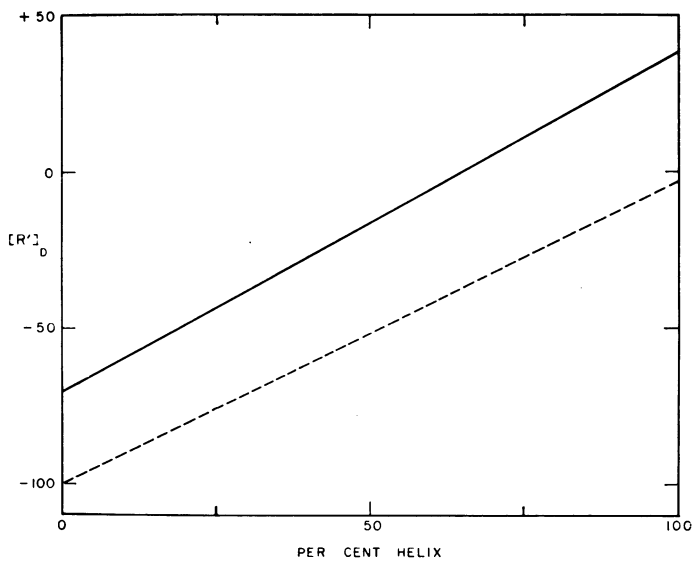

FIG. 1.—Plot of $[R']_D$ versus helix content: —— in low dielectric constant solvents; --- in high dielectric constant solvents.

interpreted as corresponding to quite different values of helix content. Therefore, it is clear that the optical rotation at the sodium D line (or at any single wavelength in the visible or near-ultraviolet) should not be interpreted in terms of helix content. It is also apparent that even small differences in effective dielectric constant, which occur between organic solvents, or with the same solvent due to differences in temperature, pH, or ionic strength give rise to variations in $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$ which lie below the experimental precision of this analysis. Such variations will give rise to fairly large differences in specific rotation at any one wavelength. Hence, differences in specific rotation at any one wavelength in the visible or near-ultraviolet do not necessarily reflect differences in helix content.[3, 4]

The extreme sensitivity of $A_{(\alpha,\rho)(193)} + A_{(\alpha,\rho)225}$ to the effective dielectric constant may, under special conditions, prove useful as a measure of change in tertiary structure or state of aggregation.

Comparison of equation (1) with the one-term Drude equation: The one-term Drude equation[5]

$$[R'] = \frac{A_c\lambda^2_c}{\lambda^2 - \lambda^2_c} \tag{5}$$

has been used in the past to represent the rotatory dispersion data of many synthetic polypeptides and proteins. It is now known that this equation represents the data for α-helical polypeptides and proteins of low helix content and even then only over a narrow spectral interval. In the cases where the one-term Drude equation fits the data, the constants A_c and λ_c are related to the constants A_i and λ_i of the multiterm Drude equation

$$[R'] = \sum_i \frac{A_i\lambda^2_i}{\lambda^2 - \lambda^2_i} \tag{6}$$

by[6] $A_c\lambda_c^2 = \sum_i A_i\lambda^2_i$ (7) $A_c\lambda_c^4 = \sum_i A_i\lambda^4_i$. (8)

Using equations (7) and (8) one can obtain from A_c and λ_c the values of $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$

$$A_{(\alpha,\rho)(193)} = +0.201 \times 10^{-8} A_c\lambda^2_c (\lambda^2_{225} - \lambda^2_c) \quad (9)$$

$$A_{(\alpha,\rho)225} = -0.148 \times 10^{-8} A_c\lambda^2_c (\lambda^2_{193} - \lambda^2_c). \quad (10)$$

If this transformation is performed for the data in the literature, it is possible to calculate, in terms of the present analysis, whether the polypeptides and proteins previously examined contain only α-helical and random conformation, and if so the approximate per cent helix. As indicated above, if the values of $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$ do not fit the appropriate equation [eq. (2) or (3) depending on the effective dielectric constant], the presence of structures other than α-helical or random may be inferred. We have carried out this calculation for a few of the polypeptides and proteins whose rotation data were previously analyzed by the modified two-term Drude equation (Table 1). However, $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$ can be more accurately determined by extending the optical rotatory dispersion measurements over a larger spectral interval and employing the modified two-term Drude equation.

TABLE 1

ROTATORY CONSTANTS FOR POLYPEPTIDES AND PROTEINS CALCULATED FROM THE ONE-TERM DRUDE EQUATION AND THE MODIFIED TWO-TERM DRUDE EQUATION

Polypeptide or protein[d]	Solvent	λ_c (mμ)	A_c	$A_{(\alpha,\rho)(193)}$ (a)	(b)	$A_{(\alpha,\rho)225}$ (c)	(b)
L-glutamic acid: L-lysine (1:1)	H_2O pH 8	235	−385	+195	+240	−560	−590
γ-morpholinyl-ethyl-L-glutamamide	H_2O pH 4. 5	200	−715	−605	−610	−115	− 90
Bovine plasma albumin	Formic acid	216	−455	−165	−140	−290	−320
Oxidized bovine plasma albumin	Formic acid	228	−360	+ 50	+ 80	−415	−420
O-acetyl-L-serine	$CHCl_3$:DCA-(3:7)	211	+625	+340	+330	+300	+340
Pepsinogen	H_2O pH 7	228	−265	+ 40	+ 60	−300	−260

[a] Calculated from equation (9).
[b] Calculated from equation (1).
[c] Calculated from equation (10).
[d] For references see preceding communications.[1, 2]

Comparison of equation (1) with the Moffitt equation: Since equation (1) and the Moffitt equation fit the rotatory dispersion data of polypeptides and proteins in the visible and near-ultraviolet, the question arises as to the relation between these two equations.

The Moffitt theory is based on the assumption that the two important contributions to the rotation in the visible and near-ultraviolet are from the 150 mμ ($N \rightarrow V_2$) and from the 190 mμ ($N \rightarrow V_1$) amide absorption bands.[7-10] Moffitt assumed further that each of these absorption bands is split into two components polarized perpendicularly and having approximately equal but opposite rotational strengths. Under these conditions the partial rotation of the $N \rightarrow V_1$ absorption band

$$[R'_1] = \frac{a_{1\parallel}\lambda^2_{1\parallel}}{\lambda^2 - \lambda^2_{1\parallel}} + \frac{a_{1\perp}\lambda^2_{1\perp}}{\lambda^2 - \lambda^2_{1\perp}} \tag{11}$$

can be rewritten as

$$[R'_1] = \frac{a_1\lambda^2_1}{\lambda^2 - \lambda^2_1} + \frac{b_1\lambda^4_1}{(\lambda^2 - \lambda^2_1)^2} \tag{12}$$

where

$$\lambda_1 = \frac{\lambda_{1\parallel} + \lambda_{1\perp}}{2},\ b_1 = (a_{1\parallel} - a_{1\perp})\Delta_1,\ \Delta_1 = \frac{\lambda_{1\parallel} - \lambda_{1\perp}}{\lambda_1},\ a_1 = a_{1\parallel} + a_{1\perp} + b_1$$

since $|\lambda^2_{1\parallel} - \lambda^2_{1\perp}| \ll |\lambda^2 - \lambda^2_{1\parallel}|$ and $|a_{1\parallel} + a_{1\perp}| \ll |a_{1\parallel}|$. The same is true for the $N \rightarrow V_2$ absorption band, and thus the total rotation can be written as

$$[R'] = \frac{a_o\lambda^2_o}{\lambda^2 - \lambda^2_o} + \frac{b_o\lambda^4_o}{(\lambda^2 - \lambda^2_o)^2} \tag{13}$$

where

$$a_o\lambda^2_o = \sum_i a_i\lambda^2_i$$

$$b_o\lambda^4_o = \sum_i b_i\lambda^4_i$$

$$b_o\lambda^6_o = \sum_i b_i\lambda^6_i$$

band if the long wavelength bands dominate. The approximations involved are such that barring other effects the fit between equation (13) and the observed data should hold to at least as short wavelengths as the modified two-term Drude equation (270 mμ).

The widely known and important equation (13), which bears Moffitt's name is based on his more general theory which predicts a value of λ_o equal to 200 mμ, and values of b_o independent of solvent varying from −580 for a completely α-helical polypeptide to zero for a completely random polypeptide. Experimentally the best fit between equation (13) and the observed rotations over the range 600–313 mμ is obtained for a value of $\lambda_o = 212 \pm 5$ mμ and gives values of b_o varying from −700 ($\lambda_o = 212$ mμ) for a completely α-helical polypeptide to a small positive value for a completely random polypeptide.[3, 4]

In contrast to the Moffitt assumptions of the importance of Cotton effects at 150 and 190 mμ, equation (1) implies Cotton effects at 193, 198, and 225 mμ. Since the physical implications of the two equations differ, the fact that both fit the rotatory dispersion data means that they must be formally equivalent.

Using the same type of transformation that yielded equation (12) from equation (11) one can rewrite the modified two-term Drude equation in the equivalent Moffitt form, where $\lambda_o = 209$ mμ and

$$\begin{aligned} b_o &= -0.153\,(A_{(\alpha,\rho)(193)} - A_{(\alpha,\rho)225}) \\ a_o &= +0.847A_{(\alpha,\rho)(193)} + 1.153A_{(\alpha,\rho)225} \end{aligned} \tag{14}$$

with the restrictions that

$$(a)\quad |\lambda^2_{193} - \lambda^2_{225}| \ll |\lambda^2 - \lambda^2_{193}| \text{ and } (b)\ |A_{(\alpha,\rho)(193)} + A_{(\alpha,\rho)225}| \ll |A_{(\alpha,\rho)(193)}|.$$

We have already shown that $A_{(\alpha,\rho)(193)} - A_{(\alpha,\rho)225}$ is a quantity nearly independent of solvent and linearly related to helix content.[2] Therefore, the same properties will be true of b_o in the region for which this transformation is valid. Since the modified two-term Drude equation fits the rotatory dispersion data to 270 mμ, any deviation of the Moffitt equation from the modified two-term Drude equation at wavelengths above 270 mμ must arise from a breakdown of the approximations (*a*) and (*b*) and not from an incorrect choice of λ_o.

As a direct method of determining the extent to which the approximations (*a*) and (*b*) are valid, we have calculated the difference between $[R']$ as given by equations (1) and (13) using the value $\lambda_o = 209$ mμ and the values of b_o and a_o given in equations (14). The data are shown in Figure 2. The difference, $\Delta[R']$, is not significant except at wavelengths close to 310 mμ and below. Therefore, a Moffitt equation with the parameters above will fit the data to about 310 mμ. An apparent extension of the region over which the equation fits can be obtained by increasing λ_o. However, such adjustment of λ_o invalidates the transformation [eqs. (14)] and hence the justification for interpreting b_o as a linear function of helix content. Thus, for values of λ_o other than 209 mμ, the linear relation of b_o to helix content has to be checked experimentally. Such a calibration necessitates

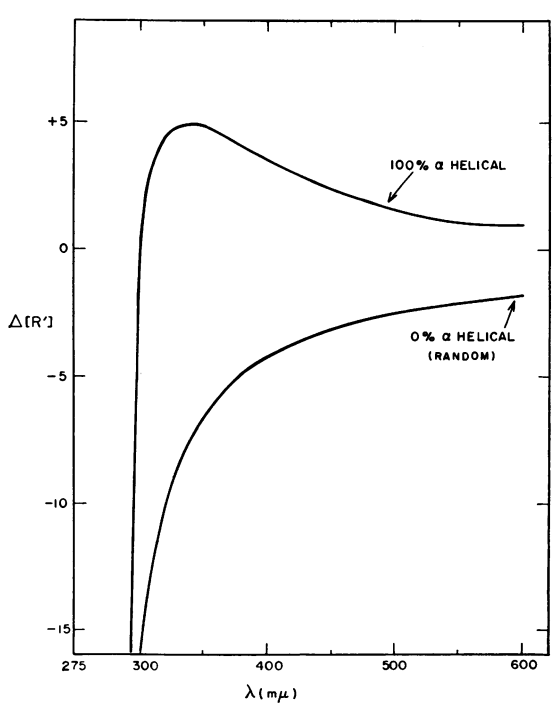

FIG. 2.—Plot of the differences in specific rotation between the modified two-term Drude equation and the Moffitt equation with $\lambda_o = 209$ mμ as a function of wavelength for two helix contents.

the use of the modified two-term Drude equation or the Moffitt equation with $\lambda_o = 209$ mμ.

The differences, $\Delta[R']$, are dependent on the per cent helix. However, in all cases, at wavelengths below 300 mμ the differences become increasingly negative. Since $[R']$ is negative, this means that a Moffitt equation with $\lambda_o = 209$ mμ and a_o and b_o as given by (14) will predict rotations which are too small in absolute value at these shorter wavelengths. Such a deviation in a Moffitt plot can be compensated by increasing the value of λ_o. Thus, as one attempts to fit the rotatory dispersion at shorter ultraviolet wavelengths, one must use larger values of λ_o.[4, 11]

From equations (14) we see that b_o and a_o are simply related to $A_{(\alpha,\rho)(193)} - A_{(\alpha,\rho)225}$ and $A_{(\alpha,\rho)(193)} + A_{(\alpha,\rho)225}$, respectively. Since we have shown that the former is solvent-independent[2] whereas the latter is strongly solvent-dependent, it is not surprising that this behavior is displayed by their counterparts b_o and a_o. As noted above for the rotation at the sodium D line, the quantity $A_{(\alpha,\rho)(193)} + A_{(\alpha,\rho)225}$ is much more sensitive to changes in effective dielectric constant than the individual coefficients $A_{(\alpha,\rho)(193)}$ and $A_{(\alpha,\rho)225}$. For this reason a_o is a highly unreliable helix content parameter, and therefore the Moffitt treatment, as suggested several years ago,[12] yields only one parameter useful in estimating helix content, namely, b_o.

By plotting the variation of b_o as given in equation (14) against helix content we find that, independent of the dielectric constant of the solvent, the value for 100 per cent helix (b_o^{100}) is -750 whereas that for zero per cent helix (b_o^0) is $+100$. Obviously these are the values one would obtain by plotting the Moffitt equation with $\lambda_o = 209$ mμ. One reason for the less negative values of b_o^{100} in the literature is that a λ_o of 212 mμ is generally used. However, we have found that the helix content calculated from a Moffitt equation with $\lambda_o = 212$ mμ and $b_o^{100} = -700$ is the same as that obtained from a calculation using $\lambda_o = 209$ mμ and $b_o^{100} = -750$. It follows therefore that the current assumption that $b_o^{100} = -630$ and $b_o^0 = 0$ for $\lambda_o = 212$ mμ leads to inaccurate estimates of helix content. To illustrate this point some of the values of helix content as calculated from the modified two-term Drude equation (equivalent to a Moffitt equation with $\lambda_o = 209$ mμ, $b_o^{100} = -750$, $b_o^0 = +100$) and from two Moffitt equations using $\lambda_o = 212$ mμ, $b_o^{100} = -700$, $b_o^0 = +100$, and $\lambda_o = 212$ mμ, $b_o^{100} = -630$, $b_o^0 = 0$ are reported in Table 2.

We have shown why λ_o has to be increased as one attempts to fit the rotatory dispersion data further into the ultraviolet. For each value of λ_o a new value of b_o^{100} has to be calculated. However, no new information can be obtained by such attempts at extending the wavelength range over which the Moffitt equation fits the optical rotatory dispersion data. It should also be clear that any attempt to obtain information from the Moffitt equation on ordered conformations other than α-helical is meaningless at present. Finally, the Moffitt equation allows the determination of only one parameter of helix content, whereas the modified two-term Drude equation leads to the calculation of two independent parameters, and thus permits the detection of other ordered structures when the relation between the two parameters deviates from that expected for a mixture of α-helical and random conformations.

TABLE 2

Polypeptide of[d]	Solvent	Per cent helix (a)	(b)	(c)
L-glutamic acid	H_2O, pH 4	100	103	115
L-glutamic acid	H_2O, pH 7	−1	−1	−16
L-glutamic acid:L-lysine (1:1)	H_2O, pH 3	58	61	62
	H_2O, pH 8	27	31	35
L-methionine	Chloroform	100	102	114
γ-benzyl-L-glutamate	Dioxane	93	95	106
	Chloroform	89	89	97
	Pyridine	76	76	81
	DCA	15	13	0
Proteins[d]				
Paramyosin	0.6 *M* KCl, pH 7	96	95	105
Myosin	" "	61	64	66
Bovine serum albumin	H_2O, pH 7	55	57	57

[a] Per cent helix calculated using the modified two-term Drude equation.
[b] Per cent helix calculated using the Moffitt equation with $\lambda_0 = 212$ mμ, $b_0^{100} = -700$, and $b_0^0 = +100$.
[c] Per cent helix calculated using the Moffitt equation with $\lambda_0 = 212$ mμ, $b_0^{100} = -630$, and $b_0^0 = 0$.
[d] For references see preceding communications.[1, 2]

From the foregoing it is clear that the Moffitt equation is an approximate form of the modified two-term Drude equation. Since the latter equation does not require the assumption of a split of the $\pi \rightarrow \pi^*$ absorption bands, the success of the Moffitt equation in representing rotatory dispersion data should not be interpreted as supporting this assumption. In this light, it is desirable to re-examine the interpretation of the far-ultraviolet absorption[13, 14] and linear dichroism[15] measurements which in the past have been considered supporting evidence for such a split, and to extend these measurements to other peptide systems.

Conclusions.—In this communication we have shown that the modified two-term Drude equation has definite advantages over methods previously available for the determination of α-helix content in solution.

The origin of the extreme sensitivity of the rotation at one wavelength in the visible or near-ultraviolet to the effective dielectric constant of the solvent has been demonstrated. This sensitivity means that changes in $[R']$ in this spectral region do not necessarily arise from changes in secondary structure and therefore that $[R']$ cannot be a reliable measure of helix content.

Since the one-term Drude equation and the Moffitt equation are approximate forms of the modified two-term Drude equation, they allow estimates of helix content. But the parameters A_c and λ_c of the one-term Drude equation can be meaningfully interpreted in terms of helix content only if they are related to the corresponding parameters of the modified two-term Drude equation. On the other hand, one can use the value of b_o obtained from the Moffitt equation as a direct helix content parameter, provided that it has been calibrated for the appropriate value of λ_o, and the sample is known to be only α-helical and random.

Hence, the advantages of the modified two-term Drude equation (1) are (*a*) that it allows more precise determinations of α-helix content by extending the range of measurements to shorter wavelengths, and (*b*) that it provides a criterion for the presence of only α-helices and random conformations in polypeptides and proteins.

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* This is Polypeptides XLVIII. For the preceding paper in this series see ref. 2.

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[2] *Ibid.*, **51**, 794 (1964).
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*POSSIBLE SOMATIC CELL MATING IN TWIN CATTLE WITH ERYTHROCYTE MOSAICISM**

BY WILLIAM H. STONE, JANIS FRIEDMAN, AND AUDREY FREGIN

DIVISION OF GENETICS, UNIVERSITY OF WISCONSIN, MADISON

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Erythrocyte mosaicism or chimerism in cattle twins is a condition in which there is a mixture of genetically different tissues forming antigenically distinct blood cells within an individual. Owen[1] proposed that vascular anastomoses between twin embryos permitted a reciprocal exchange of primordial hematopoietic tissues so that each twin possesses erythrocytes formed by its own tissues as well as those formed by tissue derived (transplanted) from its co-twin.[2-4] Presumably, tissues that give rise to histocompatibility antigens must be exchanged also, since dizygotic twins with erythrocyte mosaicism usually accept each other's skin grafts.[5, 6] Karyotypic chimerism[7] and transferrin chimerism[8, 9] may also exist. Erythrocyte mosaicism has been described in sheep,[10] humans,[11] chickens,[12] marmosets,[13] and mink.[14] It is of interest because of its significance to the phenomenon of immunologic tolerance[15] and "radiation chimerism."[16]

The proportions of the blood types in twins with erythrocyte mosaicism are essentially the same in each twin.[17-20] They may be equal in both twins or unequal so that one twin may possess more of its co-twin's blood type than of its own. The genotype of a twin can be recognized only by the blood types of its progeny.[21]

This paper presents some new observations on chimerism in cattle twins. Two phenomena have been discovered: (1) the proportion of the two cell types may change markedly with time, and (2) some kind of genetic exchange, possibly resulting from somatic cell mating, may occur between the hematopoietic tissues of the mixture yielding a cell type containing a new combination of antigens.