THE OPTICAL ROTATORY DISPERSION OF RIGHT-HANDED \( \alpha \)-HELICES IN SPERM WHALE MYOGLOBIN

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Optical rotary dispersion studies indicate that most synthetic polypeptides known to be helical in solution have an identical screw sense. In terms of the dispersion properties of these substances, this method moreover provides evidence that many proteins contain helical regions of varying extent characterized by the same handedness. 1 Although several lines of argument imply that this prevailing
sense is right-handed, the existence of right-handed helical segments in crystalline sperm whale myoglobin, as unequivocally demonstrated by Kendrew, offers a clear test of this conclusion. In addition to resolving the issue of helical sense, the rotatory dispersion of this globular protein can elucidate a second fundamental problem by virtue of the capacity of the technique to assess partial helical content. If it can show that the helical content of the crystal, which X-ray diffraction reveals to be 77 per cent, persists upon dissolving, then one will gain a convincing argument for the relevance of the crystalline structure of myoglobin to its biochemical function in solution.

The majority of helical synthetic polypeptides display complex rotatory dispersion characterized by negative \( b_0 \) values that cluster about \(-630\) when data are treated by the procedure of Moffitt and Yang and, further, exhibit a progressive levorotation as the far ultraviolet spectrum is approached. It is axiomatic that helices of opposite sense will manifest diametrically opposed rotatory properties, a principle that has been given substance in the positive \( b_0 \) values and ultraviolet rotations of poly-\( \beta \)-benzyl-L-aspartate and poly-\( \gamma \)-benzyl-D-glutamate, both of which have helical senses opposite to standard polypeptides. Which helical sense, then, right- or left-handed, gives rise to the standard rotatory dispersion?

Theories of the rotatory dispersion of helices have thus far been unable to answer this question. Although the right-handed postulate of Moffitt\(^8\) generates a negative \( b_0 \) of the correct magnitude, the critique of Moffitt, Fitts, and Kirkwood\(^9\) points to the omission of critical interactions in this exciton treatment that vitiates quantitative prediction. Fitts and Kirkwood\(^10\) adduce experimental evidence in support of their right-handed assumption, but Tinoco and Woody,\(^11\) who apply the same polarizability theory of Kirkwood\(^12\) to this problem, conclude that observed rotatory dispersions favor left-handed helices.

The first empirical correlation of optical rotatory dispersion with helical sense proceeded from the conclusion of Elliott and Malcolm\(^13\) that poly-L-alanine crystals contain right-handed helices. On the assumptions of hexagonal packing and a random arrangement of chain direction, they found that right-handed \( \alpha \)-helices made sense of the puzzling X-ray diffraction pattern while left-handed helices did not. Since all optical rotatory measurements on helical poly-L-alanine, both in the solid state and in solution, yield \( b_0 \) values that range from \(-425\) to \(-560\), this interpretation of X-ray evidence implies that negative \( b_0 \) values signify right-handed helices.

A more definitive test of this contention became possible upon Kendrew's demonstration that crystalline sperm whale myoglobin contains 118 of its 153 residues, that is, 77 per cent, in right-handed \( \alpha \)-helices.\(^2\) The side chains of L-amino acids in a right-handed helix project in a direction opposite to that of carbonyl groups hydrogen-bonded into the helix, an orientation that is in fact discerned at high resolution, so that this finding, together with a knowledge of the absolute configuration of an L-amino acid,\(^19\) establishes that the \( \alpha \)-helices in this protein crystal are right-handed.

Unfortunately, myoglobin in solution does not readily permit optical rotatory assessment of its conformation because the heme group itself dominates the dispersion over most of the visible spectrum. Early studies on both myoglobin and hemoglobin\(^21\) in fact showed that Cotton effects were associated with the char-
characteristic absorption bands of this group, a finding which suggested that its asymmetric environment endows it with strong rotatory power. At a time before the helical sense in the crystal was known, an initial attempt was accordingly made to determine the helical content of myoglobin by dispersion measurements at wavelengths higher than the heme absorption bands. Although the rotatory dispersion above 650 mμ was found still to be influenced by the adjacent Cotton effect and therefore could not be used to determine b₀, it led to a useful estimate of helical content based upon the specific rotation, [α]D. This value for the native protein was obtained by extrapolation to 589 mμ of the rotation at 750 mμ, +3°, on the ground that all helical polypeptides display almost flat dispersions in this region. A specific rotation typical of the disordered chain of myoglobin, −71°, was provided by direct measurement upon denatured globin, the heme-free derivative of myoglobin. Since complete helical content in water-soluble synthetic polypeptides is characterized by a specific rotation at 589 mμ of about 100° more positive than that for the disordered form, the observations for myoglobin placed on this scale produced an estimate of 74 per cent helix. While this value almost coincides with the subsequent precise count of Kendrew, it clearly has only an approximate significance.
In the hope that removal of the heme group might leave the secondary structure of the protein reasonably intact, rotatory dispersion measurements were then carried out on the transparent globin in the visible and near ultraviolet spectrum. Dispersion data upon globin-M in aqueous solution yield \( b_0 \) values that are compatible with a partial helical content of about 50 per cent, 23, 24 an estimate which sets a minimum for the native protein. As judged from \( b_0 \), the helical content may be increased to 80 per cent in 2-chloroethanol, a value that perhaps cannot be exceeded in view of the four proline residues in the polypeptide chain. Since this solvent raises the specific rotation at 589 m\( \mu \) from \(-15^\circ\) to \(+15^\circ\), and since the extrapolated specific rotation of myoglobin is \(+3^\circ\), it could thus be argued that the native protein has a helical content intermediate between these estimates and should indeed display a negative \( b_0 \). Nonetheless, unambiguous rotatory dispersion measurements on the native protein itself would carry greater conviction that a negative \( b_0 \) is the correlate of a right-handed helix.

Rotatory dispersion measurements on a crystalline sample of sperm whale ferri-myoglobin, 25 in 0.1 \( M \) phosphate buffer at pH 7, have more recently been made on the ultraviolet side of the visible absorption bands and the attendant Cotton effects, from 240 to 360 m\( \mu \), a region in which the peptide bond chromophores govern the dispersion. Measurements were obtained with a Rudolph 200S photoelectric polarimeter equipped with a General Electric AH-6 mercury arc. The solutions had maximum absorbance less than 2 at all wavelengths in order to avoid stray light effects that produce rotatory artifacts in regions of high absorption. 26 A comparison of the ultraviolet rotatory dispersion of native myoglobin with that for
a standard helical polypeptide of high molecular weight, a 5 per cent copolymer of L-tyrosine and L-glutamic acid at pH 4, clearly shows that the optical rotations of both are sharply levorotatory below 260 m\(\mu\), while the respective disordered forms are distinctly less levorotatory (Fig. 1). Since the dispersion for both native myoglobin and the helical polypeptide have \(b_0\) values that are large and negative, these findings establish the qualitative point that both molecules in solution possess helices of the same sense.

As a quantitative index of partial helical content, \(b_0\) for the native protein at first indicated a value of more than 90 per cent. However, the conventional treatment of these low wavelength data was brought into question by the negative curvature of the Moffitt plot in this spectral region for copoly-L-tyrosyl-L-glutamic acid in this same solvent, 0.1 \(M\) phosphate buffer (Fig. 2). A slope drawn for the helical dispersion of this polymer through low wavelength points alone would hence be considerably more negative than that fitting visible and near ultraviolet data, which gives a \(b_0\) value of \(-615\). As shown in Figure 2, the Moffitt plot for this reference polypeptide may be straightened by increasing \(\lambda_0\) from 212 to 216 m\(\mu\), an alteration that changes \(b_0\) from \(-615\) to \(-535\). Once the myoglobin data were recast with \(\lambda_0 = 216\) m\(\mu\) (Fig. 3) and \(b_0 = -535\) taken as a new scale for full helical content, a value of about 73 per cent helix was obtained from a \(b_0\) of \(-390\). This value receives support from far ultraviolet spectra of myoglobin, which indicate a helical content of 70 per cent or greater in terms of the characteristic hypochromism of the peptide bond at 190 m\(\mu\). It is of interest to note in passing that \(b_0\) for myoglobin denatured in 8 \(M\) urea, as well as the small downward curvature of the dispersion at low wavelength (Fig. 1), suggest a residual helical content of about 10 per cent, a feature that does not change upon heating in this solvent.
The quantitative agreement of helical content from rotatory dispersion with helical content in the crystal is good evidence that the secondary structure of this protein, consisting of right-handed α-helices, persists in solution. Thus, save for the unlikely contingency that myoglobin opens and then refolds into helices of opposite sense upon entering solution, the correlation of right-handed helices with a negative $b_0$ and its associated parameters is virtually certain.

If the secondary structure of crystalline myoglobin persists in solution, then it is likely that the tertiary structure of this globular protein is likewise maintained as the crystal dissolves, for any loosening of the compact tertiary structure would most probably be reflected in some loss of helical content, as appears to be the case for its heme-free derivative. The concordance of optical rotatory dispersion with the known helical content in the crystal thus offers an answer to the second important question: to what extent are structural determinations by X-ray crystallography and companion studies of amino acid sequence relevant to the functioning of proteins in solution? Since these results suggest that the intricate architecture revealed in Kendrew's crystallographic model is largely preserved in solution, one can now undertake to explain the biological activity of myoglobin in terms of a complete molecular structure.

Note added in proof: S. Beychok and E. R. Blout (J. Mol. Biol., in press) have also measured the ultraviolet rotatory dispersion of sperm whale ferrimyoglobin.

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8 Moffitt, W., these PROCEEDINGS, 42, 736 (1956).


THE GENETIC CONTROL OF ENZYME ACTIVITY DURING DIFFERENTIATION*

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There are many levels at which the process of differentiation may be considered. One particularly useful approach is based upon the premise that among the first steps in differentiation is the formation of a specialized enzymatic apparatus, which in turn produces the observable morphological and functional specialization of the various cell types. The factors which determine the particular stages at which individual enzymes are synthesized during the development of each class of cells would then play key roles in determining their ultimate differentiation. The experiments to be described demonstrate that in the case of the enzyme \( \beta \)-glucuronidase, the timing of enzyme synthesis during the development of the mouse is under the genetic control of a factor which is either closely linked to, or identical with, the locus controlling the structure of the enzyme itself.

The mutation affecting \( \beta \)-glucuronidase was originally described by Morrow, Greenspan, and Carroll\(^1\) in several inbred mouse lines, and analyzed genetically by Law \textit{et al.}\(^2\) It behaves as a simple Mendelian factor, characterized in the homozy-