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Structural origins of diamagnetic anisotropy in proteins

(protein structure/membrane structure/magnetic orientation/α helix)

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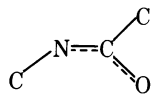
ABSTRACT **Magnetic anisotropy in proteins and polypeptides can be attributed to the diamagnetic anisotropy of the planar peptide bonds. The α helix in particular has large anisotropy due to the axial alignment of the peptide bonds. The regular arrangements of the peptide bonds in β pleated sheet and collagen structures also produce substantial anisotropy, but less than for α helix. The anisotropy permits orientation of small structures of these types in magnetic fields of several kilogauss.**

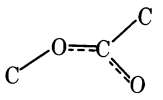
Magnetic anisotropy in biological materials has been increasingly reported. The orientation of retinal rod outer segments (1), chloroplasts (2–4), photosynthetic algae and bacteria (5, 6), purple membranes (7), and nucleic acids (8, 9) in magnetic fields of several kilogauss have been attributed to diamagnetic anisotropy of the molecular components. Earlier studies reported magnetic anisotropy in cellulosic materials (10, 11), in silks, keratins, and collagens (11), and in muscle fibers (12). The molecular origins of the diamagnetic anisotropy have been identified for only a few of these phenomena. Oriented chlorophyll molecules were proposed as the components responsible for the diamagnetic anisotropy of chloroplasts and bacterial chromatophores since the planar, partially conjugated chlorophyll ring has very large diamagnetic anisotropy (6, 13). In nucleic acids, the diamagnetic anisotropy was attributed to aromatic rings of base pairs, many of which are parallel in a DNA molecule because of the persistence length (8–9). The magnetic orientation of retinal rod outer segments was attributed to diamagnetic anisotropy of the oriented rhodopsin molecules in the disc membranes (14), but no specific molecular groups of this protein were identified to be responsible. More recently, it was proposed that oriented aromatic rings of the peptide side chains in rhodopsin could account for the magnetic anisotropy (15). Oriented lipid molecules in the disc membranes were considered not to be responsible because of the relatively weak diamagnetic anisotropy of long chain fatty acids (16) and because the orientations of these hydrocarbon chains in the lipid bilayers of the membranes would result in orientation of the opposite sense to that observed (14). Similarly, the magnetic orientation of purple membranes of *Halobacterium halobium* was attributed to the oriented molecules of bacteriorhodopsin (7), although in this case linear dichroism measurements in the region of 280 nm showed that aromatic groups cannot be responsible because their net orientation is in the wrong direction (17).

The findings of magnetic anisotropy in silks, keratins, collagens, muscle fibers, retinal rods, and purple membranes suggest that diamagnetic anisotropy is frequently present in protein structures. Oriented aromatic groups of the peptide side chains could be responsible for some of these anisotropies. However, in addition to the results for purple membranes, reports of magnetic orientation of poly(L-glutamic acid) (18), poly(L-lysine hydrobromide) (19), and poly(γ-ethyl-L-glutamate) (20, 21) suggest that there is a more fundamental basis for diamagnetic anisotropy in proteins, since these polymers of biologically occurring residues do not contain aromatic groups.

The early studies of Lonsdale (16, 22) on the diamagnetic anisotropy of organic molecules provide fundamental information concerning the molecular basis for nonaromatic origins of diamagnetic anisotropy in proteins. Lonsdale showed that "large diamagnetic anisotropy is associated with the existence of bond resonance, plane arrangement of atoms in the molecule and tendency to equalization of bond distances, not only in closed rings (as predicted by theory) but also in open systems . . . the existence of large anisotropy in a diamagnetic crystal is therefore presumptive evidence of (*i*) molecular conjugation, (*ii*) the arrangement of atoms (or the tendency of atoms to arrange themselves) in sheets normal to the direction of greatest numerical diamagnetic susceptibility" (22). On the basis of these fundamental principles, it is proposed here that diamagnetic anisotropy in proteins is due not only to oriented aromatic groups, but also to oriented peptide bonds, which are precisely the type of structure described by Lonsdale to be characteristic of diamagnetic anisotropy. The peptide bond has partial double bond character with about 30 kcal (125 kJ)/mole resonance energy and, consequently, the five atoms of the peptide bond are planar. Although no direct measurements of the diamagnetic anisotropy of the five atoms of the peptide bond have been made, it is probable that the anisotropy is nearly the same as that found by Lonsdale (16) for the five atoms of an ester bond, which also has partial double bond character, again resulting in a planar group with about 24 kcal/mole energy.



Peptide bond



Ester bond

From comparison of the anisotropy of pentaerythritol and pentaerythritol acetate, Lonsdale (16) concluded that the anisotropy in the molar diamagnetic susceptibility of an ester group is

$$\Delta K = K_{\parallel} - K_{\perp} = 8.8 \times 10^{-6},$$

where $K_{\parallel}$ and $K_{\perp}$ are the susceptibilities parallel and perpendicular to the plane of the ester group. This compares with 4.5×10^{-6} for the anisotropy of a carboxyl group, and 54×10^{-6} for the anisotropy of benzene (23).

The diamagnetic anisotropies of several types of protein structures are therefore readily understood in terms of the contributions of the planar peptide bonds. In α helices, the planar peptide bonds are oriented parallel to the helix axis,

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which therefore is the axis of smallest numerical diagmagnetic susceptibility. In β pleated sheets, the planar groups of the peptide bonds are oriented parallel to the sheet and the axis of smallest numerical diamagnetism is parallel to the pleats. In collagen, the planar peptide groups are oriented at about 45° to the fiber axis and, consequently, the axis of smallest numerical diamagnetism is perpendicular to the fiber axis. These features provide qualitative explanation for the observed magnetic orientations of several proteins and synthetic polypeptides: namely, that the α-helical structures of poly(lysine hydrobromide), poly(glutamic acid), poly(γ-ethyl-L-glutamate), α-keratins, muscle fibers, and bacteriorhodopsin orient with the α helices parallel to the magnetic field (7, 11, 12, 18–21); that silk fibroin also orients axially (11); and that collagens orient diametrically (11).

A more quantitative description is readily established by using the notation of Lonsdale (22). The contribution of a peptide bond to the susceptibility along the principal axes of the protein structure is

$$X_i = \sum_j \cos^2\Theta_{ij} K_j,$$

where the K_j are the principal susceptibilities of the peptide bond and $\cos\Theta_{ij}$ is the direction cosine of K_j with respect to X_i. For axially symmetric structures, the susceptibilities parallel ($X_\parallel$) and perpendicular ($X_\perp$) to the axis are sufficient. In addition, the peptide bond susceptibilities may be reduced to an in-plane susceptibility ($K_\parallel$) and an out-of-plane susceptibility ($K_\perp$). The equations then reduce to

$$X_\parallel = K_\perp \cos^2\phi + K_\parallel \sin^2\phi$$

$$X_\perp = [K_\parallel \sin^2\phi + K_\parallel (\cos^2\phi + 1)]/2,$$

where ϕ is the angle between the symmetry axis and the normal to the peptide planes, and $X_\perp$ has been rotationally averaged around the symmetry axis. The anisotropy is

$$\Delta X = X_\parallel - X_\perp = \Delta K\,(1\text{–}3\cos^2\phi)/2$$

for the α helix, $\phi = 90°$. For a structure containing N peptides,

$$\Delta X = (N/2)\Delta K = 4.4 \times 10^{-6} N.$$

For a β pleated sheet, $\phi \simeq 60°$ and

$$\Delta X \simeq (N/8)\Delta K = 1.1 \times 10^{-6} N.$$

For collagens, $\phi \simeq 45°$ and

$$\Delta X \simeq -(N/4)\Delta K = -2.2 \times 10^{-6} N.$$

The largest anisotropy is therefore provided by the α helix. The anisotropy given here may be compared with an experimental value of $2.6 \pm 0.2 \times 10^{-6} N$ from measurements on the cholesteric structure of liquid crystalline poly(γ-ethyl-L-glutamate) in ethyl acetate solution (21). However, this experimental value can only be regarded as a lower limit without more information on the α helix content and degree of orientation.

From the value of $4.4 \times 10^{-6} N$ for ΔX in the α helix, it follows that about 6.5 peptides contribute as much to the diamagnetic anisotropy of the α helix as one benzene ring (in phenylalanine, tyrosine, and tryptophan residues) oriented parallel to the helix axis. Similarly, one benzene ring oriented perpendicular to the α-helix axis will be offset by 13 peptide bonds. In α-helical proteins such α-keratins, muscle fibers, and bacteriorhodopsin, aromatic residues are about 10% of the total peptides. Consequently, the diamagnetic anisotropy will be dominated by the peptide backbone when there is a large percentage of parallel α helices, unless there is orientation of nearly all the aromatic rings perpendicular to the helix axis. As orientation of large numbers of the aromatic rings seems unlikely in most proteins, it should be a general property that protein structures containing substantial amounts of parallel α helices will orient with the helices parallel to the magnetic field.

In collagens, aromatic residues are only 1.6% of the total peptides, and consequently the diamagnetic anisotropy of these structures will be determined by the peptide backbone even if the aromatic groups would all contribute in the opposite sense. In β-pleated sheet structures such as silk fibroin, aromatic residues are about 10% of the peptides, and because of the much smaller contribution to ΔX from the peptide backbone (a consequence of pleating), oriented aromatic rings could often be the major contributor to the diamagnetic anisotropy.

For anisotropic structures with axial symmetry, the magnetic energy is given by

$$E(\Theta,B) = -B^2\{(X_\parallel - X_\perp)\cos^2\Theta + X_\perp\}/2,$$

where Θ is the angle between the symmetry axis and the magnetic field lines and B is the field strength. Significant orientation can occur if the difference in energy between parallel and perpendicular orientations is significant with respect to thermal energy, kT. For parallel α helices containing N peptides

$$\Delta E = (\tfrac{1}{2})B^2\Delta X = (N/4)B^2\Delta K,$$

and the magnetic energy in a field of 10 kG will be comparable to thermal energy if N is about 10^8.

This number will be greater or smaller depending on the net orientation of aromatic rings, but it is evident that very small α-helical structures can show significant orientation in magnetic fields commonly found in many laboratories. A similar number was estimated in the study of poly(L-lysine hydrobromide) (19).

The smallest α-helical structure thus far found to orient in magnetic fields is the purple membrane of *H. halobium* (7). These membranes are about 0.5 μm in diameter and 50 Å thick and consist of an in-plane lattice of lipid and protein with unit cell area of 3400 Å^2 containing three protein molecules, each with about 180 α-helical peptides oriented perpendicular to the membrane plane (24–27). Thus, there are about 3×10^6 α-helical peptides in each membrane. The distribution of orientations of these membranes in a magnetic field is given by the Boltzman distribution. The degree of orientation, expressed as the fractional difference in populations for the parallel and perpendicular orientations (7), is $1 - \exp(-\Delta E/kT)$. For these membranes in a 17-kG magnetic field, $\Delta E/kT = 0.08$, which is in good agreement with the approximately 10% orientation observed with this field strength for the membranes in dilute dispersion (7). Linear dichroism measurements in the region of 280 nm show that aromatic residues (especially tryptophan) have a small residual orientation parallel to the membranes (17) (i.e., perpendicular to the α helices) and thus will diminish the diamagnetic anisotropy contributed by the α helices. Because the degree of this diminution of ΔX is difficult to estimate, and also because there is some dispersion in the membrane sizes, the above calculation for the expected orientation is only approximate, but does demonstrate rough agreement between the theoretical and experimental anisotropy.

The description of the molecular origins of diamagnetic anisotropy in proteins presented here shows that the basic types of regular protein structures, and α helix in particular, derive substantial diamagnetic anisotropy from the nature of the polypeptide backbone. The anisotropy is sufficient to produce, in fields of about 10 kG, substantial orientation of structures as

small as 10^{-14} g, but may be increased or decreased by contributions of oriented aromatic side chains. The diamagnetic anisotropy and consequent magnetic orientation of these types of protein structures could find considerable application in diffraction and linear dichroism studies or in structural analysis of biological materials (28).

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