BRIEF COMMUNICATION

ROTATION OF SICKLE CELLS IN HOMOGENEOUS MAGNETIC FIELDS

P. COSTA RIBEIRO, M. A. DAVIDOVICH, E. WAJNBERG, G. BEMSKI, AND M. KISCHINEVSKY, *Pontifícia Universidade Católica do Rio de Janeiro, Gâvea, Rio de Janeiro, Brasil*

ABSTRACT Deoxygenated sickle cells (HbS) have been shown to orient in homogeneous magnetic fields because of magnetic anisotropy of the hemes. The time of rotation is proportional to $1/H^2$ and is in agreement with theory. Structural information concerning the orientation of HbS molecules in the fibers is obtained from these experiments and is shown to agree substantially with existing models.

INTRODUCTION

Several biological systems have been shown to orient in homogeneous magnetic fields (1–6). Such behavior results from the magnetic anisotropy of individual molecular components of the rotating body. Chloroplasts, retinal rods, nucleic acids, etc. orient themselves because of diamagnetic anisotropy (4). In the case of sickle cells it has been observed that they acquire an equilibrium orientation at 90° to the applied magnetic fields (2).

Sickle cells are erythrocytes that are deformed because of the aggregation of deoxyhemoglobin S molecules into individual fibers. Bundles of these fibers are aligned approximately parallel, deforming the cell, and resulting in its sickled appearance. The peculiar shape of the cells allows for visual observation under a microscope located in an applied magnetic field.

In the present work we study the rotation of sickle cells in such fields. We show experimentally that τ, the time of rotation, is proportional to $1/H^2$ (H-magnetic field), and from this we obtain the magnetic anisotropy of the cell. An analysis of the experimental results, based on a simple model for the fibers, leads to some structural information about the orientation of the hemoglobin molecules (HbS) in the fibers.

MATERIALS AND METHODS

A small microscope, with a X-Y micrometric table, is placed between the poles of the Varian V-1400 electromagnet (Varian Associates, Palo Alto, Calif.) that can be easily rotated around an axis perpendicular to the field. A drop of fresh blood from an HbS homozygote in a 2% solution of sodium meta bisulfite (2 parts to 1 part of blood) is placed between two microscope slides and sealed with varnish. A cell is selected in the visual field, and after its equilibrium orientation is attained, the magnet is rapidly rotated and the time τ for rotation of the cells between ϕ_i and ϕ_f is measured. ϕ_i is the angle between the long axis of the sickle cell and the magnetic field, just after the rotation of the magnet. For practical reasons ϕ_i was chosen as 35°. ϕ_f is 65°.

RESULTS AND DISCUSSION

Fig. 1 shows the experimental results. Each point is an average of 5–10 measurements on the same, as well as on different cells.

Solving the equation of motion of a rod of moment of inertia, I, in a magnetic field, $I \cdot (d^2\phi/dt^2) + \xi\,(d\phi/dt) + VH^2\,\Delta\chi\,\sin 2\phi = 0$, one obtains within the approximation $(d^2\phi/dt^2) = 0$,

$$\tau = \frac{\xi}{H^2\Delta\chi_c}\ln\frac{tg\phi_i}{tg\phi_f} \tag{1}$$

where ξ is the rotatory frictional coefficient of the cells considered as slender prolate ellipsoids, and $\Delta\chi_c$ is the cell magnetic anisotropy. The inverse proportionality between τ and H^2 is quite closely followed by the sickle cells.

Using Perrin's expression (4) for ξ:

$$\xi = \frac{16\pi\eta a^3}{6\log\left(\frac{2a}{b}\right) - 3} \tag{2}$$

with the major axis of the cell $a = 1.4 \cdot 10^{-3}$ cm, the minor axis $b = 5 \cdot 10^{-4}$ cm, the viscosity of the medium, $\eta = 1.02 \cdot 10^{-2}$ poise, we obtain $\xi = 1.92 \cdot 10^{-10}$ g – cm^2/s.

Comparison of Eq. 1 with the experimental results (Fig. 1) provides the value of $\Delta\chi_c = 2.7 \cdot 10^{-18}$ for the anisotropy of the sickle cell.

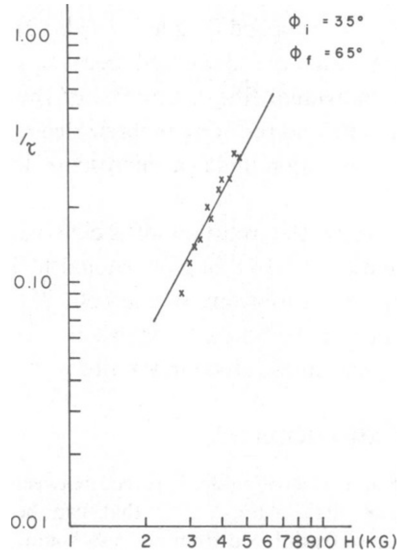

FIGURE 1 The inverse of the time of rotation τ (s^{-1}) of the sickle cells as a function of the magnetic field, H.

In all measurements ϕ_f was chosen to be 65° since it can be shown by a simple propagation of errors treatment of the equation that this final angle produces the smallest error in determining $\Delta\chi_c$.

We now relate this value of $\Delta\chi_c$ to structural parameters of the cell. Fibers exist only in deoxygenated sickle cells. The paramagnetic susceptibility of deoxyhemoglobin due to the spin state $S = 2$ of the individual hemes is, at room temperature, one order of magnitude larger than the diamagnetic susceptibility, as determined by Pauling (7). It is also known (8) that the paramagnetic susceptibility in the plane of the heme, $\chi_\perp$, is ~ 10% larger than the susceptibility perpendicular to the heme plane ($\chi_\|$). Let us then consider the effect of this paramagnetic anisotropy on the orientation of the cells.

The interaction energy of one heme with the magnetic field is

$$E = -\frac{1}{2}\Delta\chi_h(\hat{\mathbf{u}} \cdot \mathbf{H})^2 \tag{3}$$

where $\hat{\mathbf{u}}$ is the unit vector normal to the heme plane and $\Delta\chi_h = (\chi_\perp - \chi_\|)$. To calculate the magnetic energy of the whole sickled cell we make two assumptions, about the structure and alignment of the fibers in the cells, which are consistent with recently proposed models based on experimental results (9–11). These are (*a*) the HbS molecules in the fibers are oriented in such a way that the molecular *x*-axis[1] makes an angle θ with the symmetry axis of the fiber, but the molecules are otherwise randomly oriented. (*b*) The fibers, which contribute cooperatively to the magnetic orientation, are all aligned parallel to the long axis of the cell.

If we write $\hat{\mathbf{u}}$ in the molecular axis system, *xyz*,[1] as

$$\hat{\mathbf{u}} = \cos\alpha\hat{\mathbf{x}} + \cos\beta\hat{\mathbf{y}} + \cos\gamma\hat{\mathbf{z}} \tag{4}$$

the magnetic energy of the cell whose long axis makes an angle ϕ with the field **H** is given, after averaging over the molecular orientations around the fiber axis, by

$$E(\phi) = \frac{1}{2}|\Delta\chi|NH^2\frac{1}{4}\sum_{i=1}^{4}\{\cos^2\phi\,(3\cos^2\theta - 1)\,(3\cos^2\alpha_i - 1) + \cos^2\theta\,(1-3\cos^2\alpha_i) + (1 + \cos^2\alpha_i)\} \tag{5}$$

where N is the number of Hb molecules in the aligned fibers (expressed in moles). $\Delta\chi$ is the anisotropy in the molar susceptibility of the heme and α_i is the angle between the normal to the plane of the i^{th} heme group ($i = 1, 2, 3, 4$) of the molecule and the x molecular axis. Substituting the known (13, 14) values of α into Eq. 5 one obtains

$$E(\phi) = \tfrac{1}{2}H^2|\Delta\chi|N\,[1.42\cos^2\phi\,(3\cos^2\theta - 1)] + \text{terms independent of }\phi. \tag{6}$$

The sign of the coefficient of $\cos^2\phi$ is crucial to the orientational behavior of the cell. If it is positive, the ϕ-dependent part of the magnetic energy is minimized at $\phi = 90°$, whereas if it is negative it is minimized at $\phi = 0°$ or 180°. Since the experiments show that $\phi = 90°$, it is

---

[1] The y axis is the true twofold axis of symmetry of the Hb molecule, and the x and z axes are pseudo twofold axes. A rotation of 180° about the x axis maps subunit α_1 into β_1, and subunit α_2 into β_2 (10).

implied that $(3\cos^2\theta - 1) > 0$, and $\theta < 55°$; this result agrees with the existing fiber models (15, 16), based on other experiments in which $\theta < 22°$.

From Eq. 6 one can obtain an expression for the anisotropy in the molar susceptibility of the hemes as a function of θ:

$$|\Delta\chi| = \frac{\Delta\chi_c}{1.42N(3\cos^2\theta - 1)}. \tag{7}$$

Taking for the anisotropy of the whole cell, the average of all the experimental points, $\Delta\chi_c = 1.9 \cdot 10^{-18}$, and for N, the measured value of $4.32 \cdot 10^{-16}$ mol, we get

$$|\Delta\chi| = \frac{3.1 \cdot 10^{-3}}{(3\cos^2\theta - 1)}. \tag{8}$$

For $\theta = 0°$, $|\Delta\chi|$ has its minimum value of 1.55×10^{-3}. As θ increases, $|\Delta\chi|$ increases slowly, reaching at $\theta = 35°$, a value which is only about twice its minimum value. From there on it increases faster going to infinity at $\theta = 55°$. These values are to be compared to the value of 1.1×10^{-3} obtained for the paramagnetic anisotropy of the heme from another experiment (8).

The overall agreement, better for smaller θ, is gratifying, showing first that θ is most probably a small angle ($\theta < 35°$) and second, that the orientation of the cells is mainly due to the paramagnetic anisotropy of the hemes. The anisotropy in the diamagnetism of the hemes surely also plays a role in the orientation; however, its value is estimated (16) to be ~20% of the value of the paramagnetic one. To take it into account in our calculations we should add the diamagnetic energy of the hemes in the cell to Eq. 6. The paramagnetic and diamagnetic $\Delta\chi$ have both the same sign as do the energies. Therefore the diamagnetism cooperates with the paramagnetism in orienting the cells perpendicular to the magnetic field. The effect of the inclusion of the diamagnetic energy term in Eq. 6 is to bring down the calculated paramagnetic anisotropy $|\Delta\chi|$ from 1.55 to 1.29×10^{-3}, for $\theta = 0$, leading to a better agreement with the value obtained in reference 15.

The α-helix has also been shown (6) to have diamagnetic anisotropy, because of axial alignment of the planar peptide bonds. In the Hb molecule, however, they seem to be nearly randomly oriented, in which case they do not contribute to the magnetic anisotropy.

From these experiments we have obtained limits to the value of θ but we have not been able to get an exact value for it. No value of θ was consistent with the known value of the heme paramagnetic anisotropy (15). One of the possible reasons for this is the simplfying assumption that all fibers in the cell are parallel. In fact it is known (13) that there are fibers, or groups of fibers that are not aligned parallel to the long axis of the cell. The contribution of these fibers to the magnetic anisotropy is difficult to estimate because of the lack of cylindrical symmetry. Another reason is the possible error in the value of the rotatory frictional coefficient since the geometry of Perrin's formula does not correspond exactly to the actual shape of the sickle cell.

We should finally point out that the present method of orientation is very convenient for biological systems because it has apparently no destructive effect and it allows the study of properties of these systems in their natural physiological environment.

We thank the Instituto de Hematologia (Rio de Janeiro).

Received for publication 30 April 1981.

REFERENCES

1. Arnold, W., R. Steele, and H. Mueller. 1958. On the magnetic asymmetry of muscle fibers. *Proc. Natl. Acad. Sci. U. S. A.* 44:1-10.
2. Murayama, M. 1966. Molecular mechanism of red cell "Sickling." *Science (Wash., D.C.)* 153:145–149.
3. Chalazonitis, N., R. Chagneux, and A. Arvanitaki. 1970. Rotation des segments externes des photorécepteurs dans le champ magnétique constant. *Acad. Sci. Paris, D.* 271:130–133.
4. Hong, F. T., D. Mauzerall, and A. Manro. 1971. Magnetic anisotropy and the orientation of retinal rods in a homogeneous magnetic field. *Proc. Natl. Acad. Sci. U. S. A.* 68:1283–1285.
5. Chabre, M. 1978. Diamagnetic anisotropy and orientation of α helix in frog rhodopsin and meta II intermediate. *Proc. Natl. Acad. Sci. U. S. A.* 75:5471-5474.
6. Worcester, D. L. 1978. Structural origins of diamagnetic anisotropy in proteins. *Proc. Natl. Acad. Sci. U. S. A.* 75:5475–5477.
7. Pauling, L., and C. Coryell. 1936. The magnetic properties and structure of hemoglobin, oxyhemoglobin and carbonmonoxy hemoglobin. *Proc. Natl. Acad. Sci. U. S. A.* 22:210–216.
8. Nakano, N., J. Otsuka, and A. Tasaki. 1972. Paramagnetic anisotropy measurements on a single crystal of deoxyhemoglobin. *Biochem. Biophys. Acta.* 278:355–371.
9. Wilson, S. M., and M. W. Makinen. 1980. Electron microscope study of the kinetics of the fiber to crystal transition of sickle cell hemoglobin. *Proc. Natl. Acad. Sci. U. S. A.* 77:944–948.
10. Wellems, T. E., and R. Josephs. 1979. Crystallization of deoxyhemoglobin S by fiber alignment and fusion. *J. Mol. Biol.* 135:651–674.
11. Dykes, G., R. H. Crepeau, and S. J. Edelstein. 1978. Three dimensional reconstruction of the fibers of sickle cell haemoglobin. *Nature (Lond.).* 272:506–510.
12. Dickerson, R., and I. Geis. 1969. The Structure and Action of Proteins. W. A. Benjamin, Inc. 56.
13. Perutz, M., and G. Fermi. Bank of Protein Data 4/75.
14. Bernstein, F. C., T. F. Koetzle, G. J. B. Williams, E. F. Meyer, M. D. Brice, J. R. Rodgers, O. Kennard, T. Shimanouchi, and M. Tasumi. 1977. The protein data Bank: A computer based archival file for macromolecular structures. *J. Mol. Biol.* 112:535–542.
15. Hofrichter, J., D. Hendricker, and W. Eaton. 1973. Structure of hemoglobin S fibers. Optical determination of the molecular orientation in sickled erythrocytes. *Proc. Natl. Acad. Sci. U. S. A.* 70:3604–3608.
16. Wishner, B. C., K. B. Ward, E. E. Lattman, and W. E. Love. 1975. Crystal structure of sickle-cell deoxyhemoglobin at 5Å resolution. *J. Mol. Biol.* 98:179–194.
17. Knox, R., and M. Davidovich. 1978. Theory of fluorescence polarization in magnetically oriented photosynthetic systems. *Biophys. J.* 24:689–712.