LETTERS TO NATURE

Crystallization of Reconstituted Sperm Whale Myoglobins

WE wish to report that reconstituted sperm whale myoglobin prepared by the method of Breslow[1] (except that *p*H 2 was found sufficient to remove all the haem) (I) crystallizes[2] in a different habit from those prepared by the method of Rossi-Fanelli *et al.*[3] (II) using haemin of Sigma lot 77B-0220 and our own ^{57}Fe photoporphyrin preparation and the native myoglobin (III). Although all three form type A[3] monoclinic prisms, the best developed plane is [001] for II and III, it is [100] for I. There seems to be great interest in reconstituted haemoproteins[4,5], so it is important that crystallization habit may be a sensitive test for subtle changes in protein structures.

The most accurate proof for the difference in habit is obtained from the electron paramagnetic resonance (EPR) data. Kendrew and Parrish[2] report that for III below *p*H 7 one obtains monoclinic crystals flattened in the [001] plane; above *p*H 7, in the [100] plane. The *ac** plane in the two types of crystals is the same. The *g* value along an arbitrary direction α is given by[6]

$$(g^2)\alpha = g_\perp^2 [\cos^2(X, \alpha) + \cos^2(y, \alpha)] + g_\parallel^2 \cos^2(z, \alpha)$$

where *z* is the haem normal and $g_\perp = 6$, $g_\parallel = 2$ for metmyoglobin. The calculated and observed values of *g* for the two axes in crystal I agree within experimental error (Table 1). This may be taken to mean that no detectable (< 2°) changes in the haem plane orientation are effected by whatever changes the crystal habit.

Table 1 *g* Values at the *a** and *b* Axes in Reconstituted Myoglobin I

Axis	$g_{experiment}$ α	$g_{calculated}$ α
*a**	2.92 ± 0.03	2.86 ± 0.11
b	5.50 ± 0.03	5.64 ± 0.13

Other evidence which supports the above conclusion is the following. Microscopic measurements, for which the crystal was mounted on a glass capillary and examined with a low power, rotating stage, polarizing microscope, show that the two crystals have the same morphology (Table 2). The visible spectra of solutions of I, II, and III are in good agreement. The ratios of relative absorbances at 4095, 5050, and 6300 Å agree within 1%. It should be noted that there are very weak features in all the samples at 5420 and 5780 Å. These features are slightly more pronounced in the reconstituted species. These do not seem to be removed by ferricyanide oxidation or fractionation on 'Sephadex'. The reconstituted protein (I and II) oxygenate to give the characteristic spectrum of oxymyoglobin.

The importance of this observation lies in the unavoidable conclusion that I must differ from II and III in some way which has escaped detection so far. A likely possibility would be the masking of one or more of the six titratable residues of the globin so that the *p*K of the residue is lowered. Breslow[1] detected no difference when native and reconstituted myoglobins were titrated. But most titrations of this type seem to result in an error of ±1 protonation. A more refined technique is the n.m.r. difference spectra[7]. Comparison of I, II and III by Fourier transform difference n.m.r. spectroscopy is under way in this laboratory. Whereas in II and III, the residue could be protonated at *p*H 7 and some growth characteristic of the crystal faces changed at this *p*H, this change does not occur in I.

Table 2 Interfacial Angles of Myoglobins Crystals

Angle	Habit (flattened plane) [001]	[100]	From cell constant
([110], [1$\bar{1}$0])	137 ± 5	139 ± 5	128 ± 2
([100], [1$\bar{1}$0])	113 ± 5	115 ± 5	116 ± 2
([001], [100])	105 ± 1	105 ± 1	105.5

Finally, the same difference in crystallization behaviour was also observed with proteins reconstituted from cobaltous protoporphyrin IX and the two apomyoglobins. A report on the EPR spectra of single crystals of these cobalt myoglobins will be published shortly.

L. C. DICKINSON
J. C. W. CHIEN

Department of Chemistry,
University of Massachusetts,
Amherst,
Massachusetts 01002

[1] Breslow, E., *J. Biol. Chem.*, **239**, 476 (1964).
[2] Kendrew, J. C., and Parrish, R. G., *Proc. Roy. Soc.* (London), A,**238**, 305 (1957).
[3] Rossi-Fanelli, A., Antonini, E., and Caputo, A., *Biochim. Biophys. Acta*, **30**, 608 (1958).
[4] Yonetani, A., Drott, H. R., Leigh, J. S., Reed, G. H., Waterman, M. R., and Asakura, T., *J. Biol. Chem.*, **245** (11), 2998 (1970).
[5] Shulman, R. G., Wutherich, K., Yamane, I., Antonini, E., and Brunori, A., *Proc. US Nat. Acad. Sci.*, **63**, 623 (1969).
[6] Schonland, D. S., *Proc. Phys. Soc.*, **73**, 788 (1959).
[7] King, N. L. R., and Bradbury, J. H., *Nature*, **229**, 404 (1971).

Structure of the "Peroxy-Y Base" from Liver tRNA^{Phe}

THE proposed[1] structure I for the fluorescent Y base isolated[2,3] from baker's yeast tRNA^{Phe} has received support from independent data by Thiebe *et al.*[4], and been established by total synthesis; the configuration of the chiral centre has also been established as L (ref. 4*a*). The very closely related structure IV has been assigned to the "Y" base from brewer's yeast[5].

We report results of structural studies on the fluorescent base isolated from the tRNA^{Phe} of rat[6], beef[7], calf and chicken livers. The location of this base, which we designate "peroxy-