THE CRYSTAL STRUCTURE OF FERRIMYCOBACTIN P,
A GROWTH FACTOR FOR THE *MYCOBACTERIA*

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Abstract

An X-ray study of the crystal structure of ferrimycobactin P has confirmed in detail Snow's structure and absolute stereochemistry for mycobactin P and has identified the iron coordination pattern. The ferric ion lies exposed in a splayed V-shaped cleft, and is coordinated by five oxygens and one nitrogen in a very strained octahedron whose dimensions offer insight into both the exceptionally high stability of the complex and the ease of release of the iron atom.

The mycobactins, a family of iron(III) chelating compounds, are produced with minor structural variations by most species of *Mycobacteria*, and act as growth factors.(1,2) As production of mycobactin is stimulated by iron deficiency in the culture medium it was originally thought to act as a ferric ion scavenger in the culture medium.(2) Recently, however, Ratledge *et al.*, (3,4) alerted by the very low solubility of ferri- and desferrimycobactins in both water and cytoplasm, have carried out tracer feeding experiments which indicate that they are located in the lipid-rich regions of the cell wall, and that they participate in a concentration controlled diffusion cycle to transport iron through the cell wall. They are thought to capture Fe^{3+} ions at the outer surface and to release them to cytoplasm, probably *via* an NADH-dependent reduction to Fe^{2+}, which has little affinity for mycobactin. The ease with which this occurs is all the more remarkable in view of the extremely high pK_s value (> 30) reported for ferrimycobactin.

The existence of these growth factors was first inferred in 1912, and mycobactin P (from *M. phlei*) was first isolated in 1946 as a crystalline aluminium complex. Metal-free mycobactin P was obtained in 1953 and several other metal complexes were prepared. Snow has recently published a comprehensive review of the mycobactins (2) and has shown that their structures are variants of (I). Mycobactin P has the substituents and chiralities in (Ia). He was able to make eight models of the possible iron coordination, and he preferred three for their compactness and lack of strain. The present work was undertaken to identify which coordination occurs.

The first attempts to crystallise ferrimycobactin P gave very thin laths showing parallel extinction. A colleague, Dr. F.H. Allen, determined the unit cell and spacegroup, and demonstrated the isomorphism of the iron and aluminium complexes, but the size and quality of the crystals precluded further work.

Later, after considerable effort, Dr. Snow and his colleague, Mr. White, produced larger, better textured crystals from aqueous methanol at 3°C, and these have proved stable to air and to X-rays. Crystallographic data: $C_{47}H_{72}N_5O_{10}Fe$, M = 922.9, a = 16.061(1), b = 12.197(1), c = 13.282(2) Å , β = 101.39(2)°; V = 2550.6 $Å^3$; D_m = 1.201, D_c = 1.201 g cm^{-3} for Z = 2. Systematic absences and optical activity identify the spacegroup as $P2_1$.

The first attempts to solve the structure were based on a unique set of 2830 observed reflections collected on a Siemens automatic diffractometer used with Cu-K_α radiation. Neither the heavy-atom approach nor the use of direct methods revealed enough atoms to permit the structure to be recognised. It was eventually solved by making use of the considerable anomalous scattering of Cu-K_α radiation by the iron atom. A fresh set of measurements on the diffractometer gave 5240 observed reflections in the +l hemisphere of radius corresponding to θ = 55°. The intensities of the Bijvoet pairs were used to calculate phases for 2197 reflections, and to these were added all the $h0l$ reflections that could be phased reliably from the iron positions, making a total of 2477 reflections with known phases.

The corresponding Fourier summation yielded 41 atoms that made chemical sense, and the next ρ and $\Delta\rho$ maps yielded all but the last 6 atoms of the aliphatic chain. Comparison with Snow's structure enabled all the atom types to be identified, and least-squares refinement on all data (with Fe anisotropic and all other atoms isotropic) gave *R* 0.137. All 57 atoms refined smoothly, though the *B* factors increased rapidly as one went out along the aliphatic chain.

Figures 1 and 2, which depict the absolute configuration of the structure found, show that the whole of Snow's organic structure (Ia) is correct including the chiralities at all dissymmetric atoms.* The molecule of ferrimycobactin P is, apart from its aliphatic chain, roughly spherical with an effective diameter of ∿ 11 to 14 Å.

The coordination of the ferric ion is effected by the same six atoms, *a*...*f*, that Snow chose and follows his model 5 (see Fig. 8, *p*.163 of ref. 1), one of the favoured three. Figures 1 and 2, however, show that the octahedral coordination by five oxygens and one nitrogen is badly strained (nonchelate angles range from 85 to 105°). The ferric ion lies exposed in a V-shaped cleft and is flanked by four oxygen atoms, *a*,*c*,*e*,*f*. The cleft is splayed wide open (a - Fe - f = 103°; c - Fe - e = 192°), and the non-bonded gap O_a - O_f is 3.11 Å, considerably larger than all the other edges of the octahedron. The ligand bond lengths vary widely but are not yet fully refined. Atoms *a*, *c*, *e* are all negatively charged, but whereas the bonds to *a*, *e* are short (1.92, 1.91 Å) that to *c* is long (2.11 Å). Atom *e* appears to be involved in a NH...O hydrogen bond (3.00 Å) which may help strain the c - Fe - e axis. It is noteworthy that, though the axes a - Fe - d and c - Fe - e are far from straight, the sums of the *trans* bonds are 4.05 and

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* In (Ia) we have corrected a drawing error in Snow's formula (II), *p*162 of ref. 1. This was at the meeting point of all three chains which should have corresponded to Snow's mention of the isolation of L-N^6 hydroxylysine from this part of the molecule. We have also incorporated a minor revision revealed by the X-ray study in the relative orientations of the *cis* double bond in the aliphatic chain and of the adjacent hydroxamic acid group.

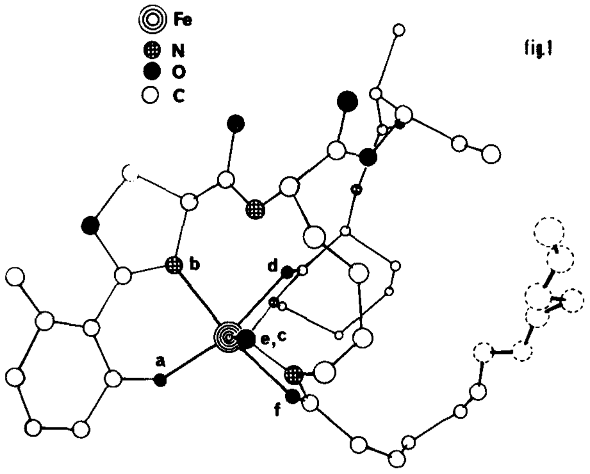


fig.1

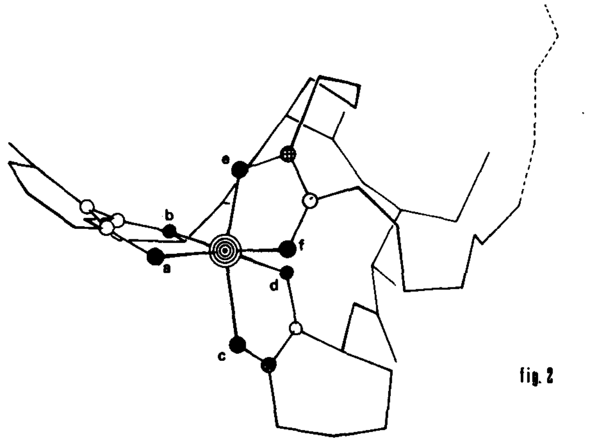
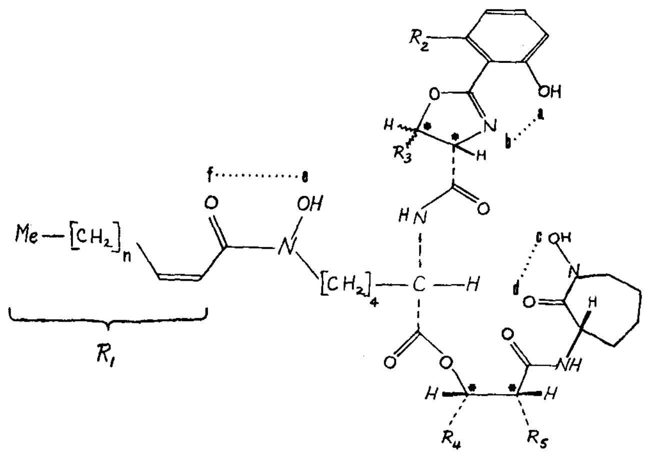


fig.2

4.02 Å respectively; there is thus a marked *trans*-effect in each case. There are marked differences in the ligand strengths of the two hydroxamic acid groups: that conjugated to the *cis* double bond is much the more tightly bound, partly due to the NH...O$_e$ bond and partly due to the conjugation. In addition, had the double bond not occurred where it does, access to the iron atom would have been partly blocked. The present results are generally in line with conclusions recently reached from Mössbauer studies of ^{57}Fe-mycobactin P.(5) Apart from one other NH...O hydrogen bond (2.81 Å),which occurs between atoms in the rear of the molecule, there appear to be no other, either intra or intermolecular hydrogen bonds. The increase of *B*-factors along the



(I) R_1, R_2, R_3, R_4, R_5 VARY IN DIFFERENT MYCOBACTINS AND
* INDICATES CENTRES OF VARYING CHIRALITY.

MYCOBACTIN P (IA) HAS R_2 = Me, R_3 = H, R_4 = Et, R_5 = Me.
n (IN R_1) = 16, 14, 12, WITH 14 PREPONDERANT.
a...f DENOTE THE COORDINATING ATOMS.

chain may be due to increase of thermal motion, and/or disorder, and/or the incorporation of molecules of assorted chain length.

The occurrence of one nitrogen in the coordination octahedron and the dissimilarities in the three chelating groups distinguish the mycobactins from the sideramines. Mycobactin-like substances have also been isolated from the *Nocardia*, (6) and seem to play a similar role. These organisms also have lipid containing cell walls, which is a comparative rarity among bacteria.

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