X-ray Photoelectron and Electron Spin Resonance Spectra of Iron(III) Parabactin

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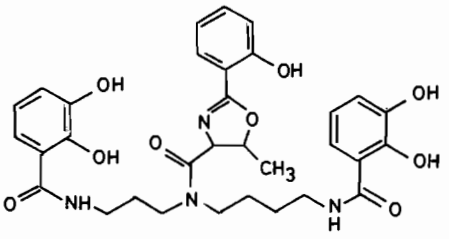
In 1979, Neilands [1] described the structure of parabactin, a high-affinity iron chelator (siderophore) from *Paracoccus denitrificans*. Neilands' report was subsequent to the discovery by Tait [2] of the siderophores of *Paracoccus*. Parabactin is shown in Fig. 1a. Nuclear magnetic resonance spectroscopy of parabactin and the closely related siderophore agrobactin [3] and the X-ray crystal structure of agrobactin [4] leave little uncertainty about the structure of parabactin.

Neilands also has performed titration experiments which have determined that five protons are released by parabactin upon complexation of iron [5]. Electrophoretic mobility of the iron complex indicates that it is a dianion. These experiments give direct evidence that the five phenyl hydroxyl oxygens are coordinated to iron. These experiments and examination of the CPK models led Neilands to conclude that the oxazoline nitrogen is the sixth ligand for iron. As further evidence of oxazoline nitrogen coordination to iron, van der Helm [4] cites the facts that the ferric parabactin complex exists as the Λ-*cis* isomer and that ferric mycobactin (Fig. 1b) is known through X-ray crystallography to employ oxazoline nitrogen coordination to iron [6]. However, optical activity in the iron complex does not even require a tris-bidentate mode of complexation [7] and certainly does not implicate oxazoline nitrogen as a ligand of iron. Thus, there is no direct evidence that the oxazoline nitrogen of parabactin is coordinated to iron. Authors of more recent papers mistakenly have accepted as fact the possibility that oxazoline nitrogen may be bound to iron [8]. The purpose of this communication is to emphasize that the sixth ligand of iron(III) parabactin has not been determined and to present some evidence that the sixth ligand might not be the oxazoline nitrogen.

X-ray photoelectron spectroscopy in the nitrogen 1s region (400 eV) is a direct test of the proposed nitrogen–iron bond in ferric parabactin. If nitrogen does bind iron, electron donation from nitrogen to iron would result in a decrease of electron density at nitrogen, partial oxidation of nitrogen and an increase in the energy with which the nitrogen 1s electrons are bound to the nucleus. This report contains our measurement of the nitrogen 1s spectra of parabactin, ferric parabactin, and for comparison, mycobactin and ferric mycobactin. We also report the electron spin resonance spectrum of ferric parabactin.



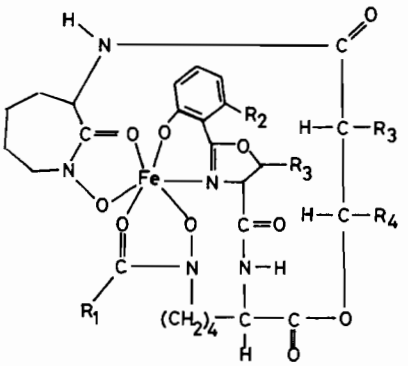


Fig. 1. (a) Parabactin, (b) ferric mycobactin. R_1 through R_5 vary depending upon the species of *Mycobacteria*.

Experimental

Paracoccus denitrificans was grown as described [2]. Parabactin was isolated, and parabactin and ferric parabactin were purified as described [9]. Parabactin and ferric parabactin are clearly separated from other catechol containing species by the purification procedures employed.

Mycobacterium smegmatis was grown as described [10]. The cells were harvested by centrifugation at 16,300 × g in a Sorvall RC2-B centrifuge. The cells were stirred with 95% ethanol for 24 h and filtered to remove the cells. Ferric chloride in ethanol was added to the filtrate until no further increase in red-brown color was apparent. The ethanol was removed by rotary evaporation and the residue dis-

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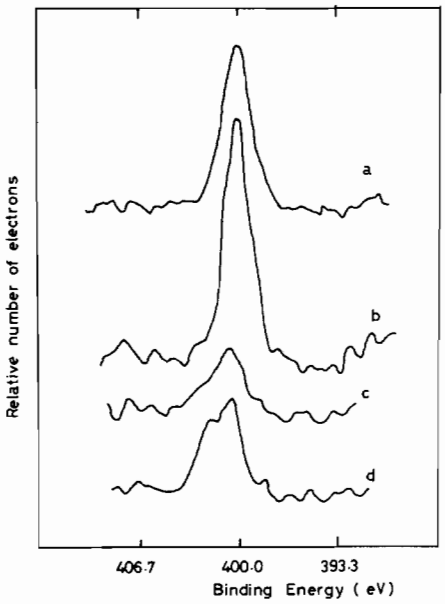

Fig. 2. Nitrogen 1s X-ray photoelectron spectra. (a) Parabactin, (b) ferric parabactin, (c) mycobactin, (d) ferric mycobactin.

solved in chloroform. The chloroform was washed with water several times and the aqueous phases were discarded. The chloroform was removed by rotary evaporation and the residue suspended in a mimimum amount of chloroform and streaked on a silica gel G thin layer chromatography plate. The plate was developed in 2:3:3 petroleum ether:ethyl acetate: n-butanol. The ferric mycobactin was eluted with methanol, filtered, and the filtrate was reduced to a minimum volume by rotary evaporation. Ferric mycobactin was chromatographed on a Sephadex LH-20 column in methanol. Ferric mycobactin was dissociated to yield pure mycobactin as described [11].

Samples for X-ray photoelectron spectroscopy were mixed with a polybutadiene adhesive and applied as a thin layer to a stainless steel sample holder using a 1% solution of trichloroethylene. The apparatus used in these experiments has been described [12]. The instrument was calibrated against the two 4f lines of gold. The resolution of the apparatus is 1 eV. The electron spin resonance spectrum was recorded at 4 K as previously described [13].

Results and Discussion

Figure 2 presents the X-ray photoelectron spectra in the nitrogen 1s region for both free ligands and both iron complexes. A single peak is observed at 400.0 eV for parabactin and for ferric parabactin. Mycobactin shows a single peak at 400.7 eV but

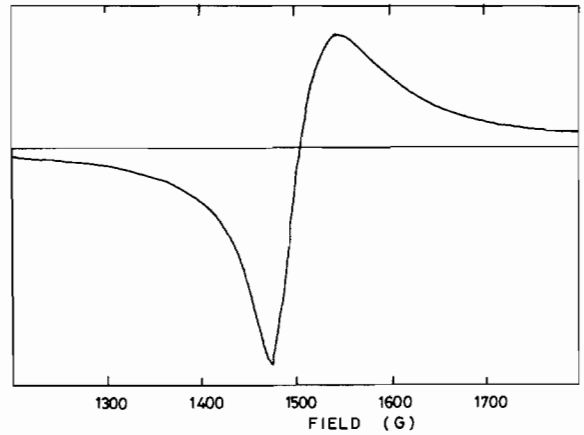

Fig. 3. Electron spin resonance spectrum of ferric parabactin.

ferric mycobactin clearly shows two peaks, one at 400.3 eV and the other at 402.0 eV. Binding of the oxazoline nitrogen in ferric mycobactin is demonstrated by the shift to higher binding energy of the nitrogen 1s electrons. No such shift is seen in the parabactin and ferric parabactin spectra, however. The most obvious explanation is that the oxazoline nitrogen of parabactin is not a ligand of iron. It is also possible that the parabactin oxazoline nitrogen is a ligand of iron but that the shift in the 1s electron binding energy is less than the resolution of the instrument. The shift in binding energy in fact may be less due to the expected increase in electron density at the iron in ferric parabactin compared to ferric mycobactin. The iron in ferric mycobactin is coordinated to three anions to yield a neutral complex, but the iron in ferric parabactin is most likely surrounded by five anions to yield a complex of charge minus two [5]. The iron in ferric parabactin may be less oxidizing and the nitrogen to iron electron donation correspondingly weaker. This also would imply a weaker, longer iron-nitrogen bond than is found in ferric mycobactin. A weak bond to iron is difficult to reconcile in view of the statement that the affinity for iron(III) of parabactin is closely comparable to that of enterobactin [5] which has a measured formation constant of 10^{52} [14].

The electron spin resonance spectrum of ferric parabactin (Fig. 3) exhibits the g = 4.28 signal characteristic of high-spin iron(III) in a rhombically-distorted octahedral field [15]. This signal is seen in a number of ferric siderophore complexes [16]. A five-coordinate ferric parabactin complex is thereby ruled out (as is a seven-coordinate complex).

In summary, reports in the literature seem to establish a coordinate bond between oxazoline nitrogen and iron in ferric parabactin, but in fact no direct evidence exists to support that assignment. The X-ray photoelectron spectra presented herein strongly indicate that the oxazoline nitrogen is not

a ligand of iron, but this data cannot be regarded as conclusive. The ESR spectrum of ferric parabactin establishes a rhombically-distorted octahedral environmental for high-spin iron(III) in ferric parabactin.

Acknowledgements

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