IDENTIFICATION OF IRON (II) ENTEROBACTIN AND ITS POSSIBLE ROLE IN *ESCHERICHIA COLI* IRON TRANSPORT

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1. Introduction

The observation that microbial iron compounds (siderophores) share the same receptor as phages and colicins on the surface of *Escherichia coli* cells [1,2] has initiated considerable research effort into the isolation and mode of action of these outer membrane proteins [3–5]. In contrast, the mechanism for iron translocation across the cytoplasmic membrane (inner membrane) has received relatively little attention. It has been stated that iron permeates the cytoplasmic membrane as iron (III) enterobactin (fig.1a) and that iron is released intracellularly [6,7]. The external face of the cytoplasmic membrane is positive with respect to the inside [8] and the movement of iron enterobactin is dependent on this membrane potential [9]. This presents an interesting problem in that, in contrast to many iron siderophores which are neutral complexes, the ferric enterobactin anion carries three charges in the pH range 6–9 [10], and this anion would appear to have to move across the membrane from the positive to the negative side.

Here we report the isolation of iron (II)-enterobactin. The existence of this complex may well have

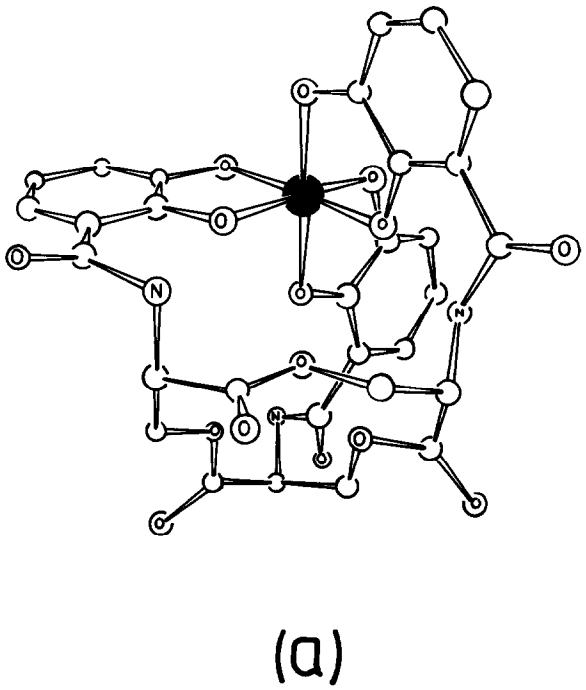
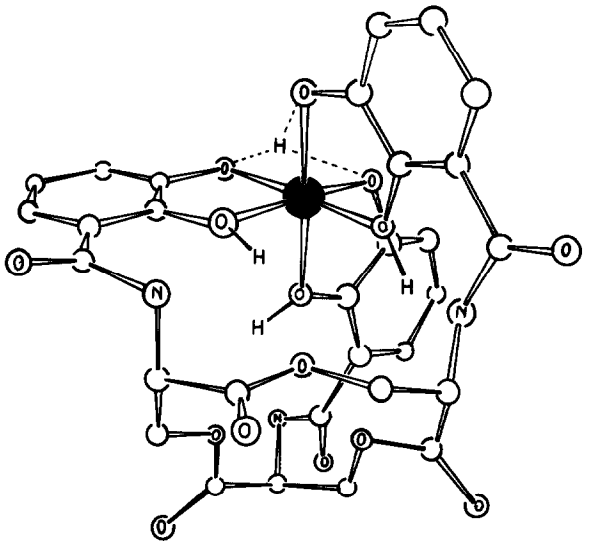


Fig.1. (a) Iron (III)–enterobactin, the major form at neutral pH values.



(b)

Fig.1. (b) Iron (II)–enterobactin, the major form at pH 4.0.

some relevance to the mode of iron–enterobactin translocation across the cytoplasmic membrane.

2. Methods

Mössbauer measurements were performed in frozen methanol solution at 80 K, using a 10 mCi ^{57}Fe source. The spectra were computer fitted.

Electronic spectra were performed at 293 K using a Perkin Elmer '575'.

'pH' measurements in methanol solutions were made with a glass electrode and the proton concentration was adjusted by the addition of a methanolic solution of Tris-free base. The pH meter gave reproducible responses under these conditions; but the response time was slower than in aqueous solutions.

3. Results and discussion

From potentiometric measurements it is clear that in the pH range 4–6, the charged state of the iron–enterobactin complex changes [10]. The charge modification coincides with an alteration in the electronic spectra seen in the visable region as a change from blue to wine with increasing pH (fig.2). A sharp colour change takes place at pH 3.9 in dry methanol solutions. This type of colour change is also seen in model systems for enterobactin [11,12]. In the iron–catechol system, at low pH, a green colour is observed; at high pH the complex is red [11,12].

Mössbauer studies of the green catechol complex show the iron to be present as iron (II) [11]. To date, the only Mössbauer study on iron–enterobactin [13] was carried out on products obtained at pH 7.0 (in methanol); the results of this work showed the iron to be present as iron (III) and the colour of this material at pH 7.0 was wine.

We now report the preliminary results of a continuing Mössbauer investigation on iron–enterobactin and related systems. The Mössbauer spectrum shown in fig.3 indicates that iron–enterobactin at pH ~4

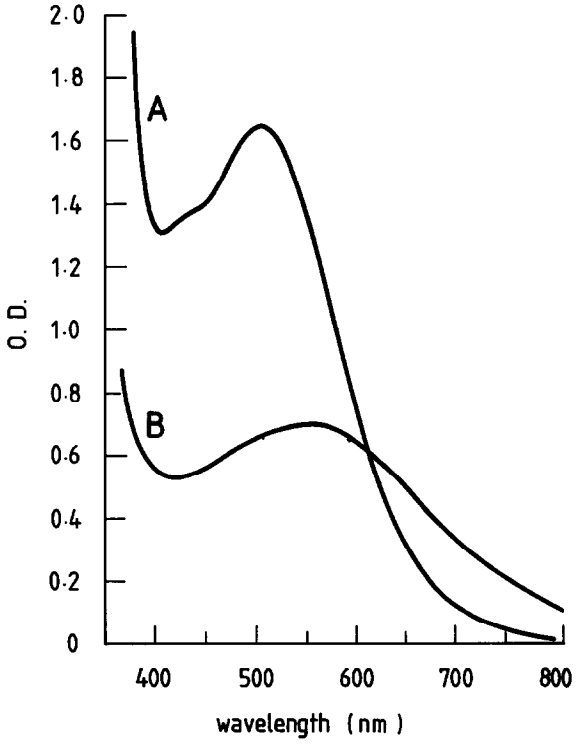


Fig.2. Electronic spectra of iron enterobactin in methanol. (A) Wine complex pH 7.0. (B) Blue complex pH 4.0. A similar transition occurs in aqueous media.

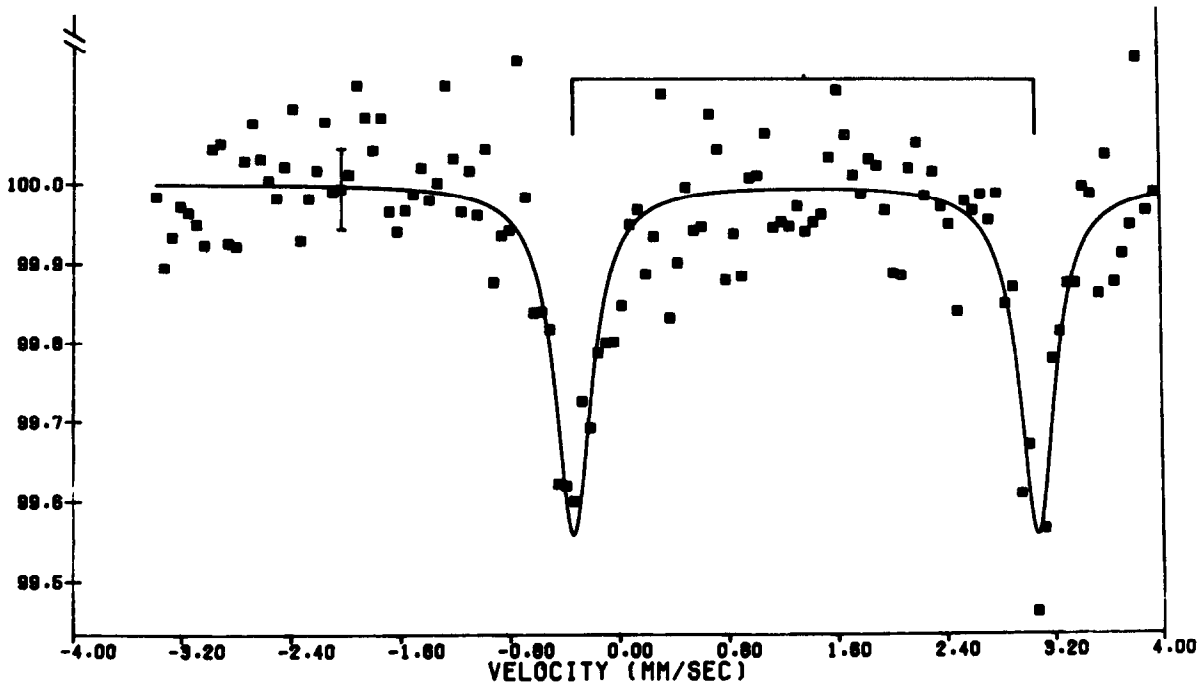


Fig.3. Mössbauer spectrum of iron–enterobactin in methanol at pH 4.0 at 80 K. The chemical isomer shift (δ) = 1.37 (1) mms^{-1}; the quadrupole splitting (Δ) = 3.41 (2) mms^{-1}; the line-width (Γ) = 0.16 (2) mms^{-1}.

(in methanol) contains iron (II). The chemical shift of 1.37 mms^{-1} is clearly iron (II) and the quadrupole splitting of 3.41 mms^{-1} is indicative of iron (II) in a slightly distorted octahedral environment. The formation of the iron (II) complex (fig.1b) probably occurs through an electron transfer process by interaction of the iron centre with the catechol rings and possibly adjacent water molecules. This redox process is reversible. The exact stereochemistry of the iron (II)–enterobactin complex reported here is not yet fully established. However, as it does not react with thiocyanate or ferricyanide, the iron (II) is probably still hexa-coordinated to the enterobactin through the 6 catechol oxygens as in the iron III structure [10,14,15]. This is in keeping with our variable pH experiments in methanol where the intense blue caused by such a species changed to light purple when water is added. This is because water competes with the catecholic rings at low pH showing that the stability constant of the iron (II)–enterobactin complex is relatively small compared to the red iron (III)–enterobactin complex which, from inference to the similar tricatechol species, has a stability constant $> 10^{45}$ [12] and does not change colour on addition of water to the red methanol solution. From the iron–enterobactin/proton titration curve [10], iron (II)–enterobactin at pH 4.0 would be predicted to possess 4 additional protons compared to the iron(III)–enterobactin anion at pH 7.0. These extra protons are associated with the 6 catechol oxygens which bond dative covalently to iron (II) (fig.1b).

It was reported [17] that complex formation between iron (III) and catechol is followed by redox reactions in the pH range 1–2. However, the iron (II) state is only considered by them to be an intermediate in the redox process.

The environment between the cell wall and the cytoplasmic membrane is likely to be acidic due to the activity of the various H^+-translocation systems located in the cytoplasmic membrane [17]. Thus a proportion of the iron–enterobactin in this intermembrane space would be expected to exist in the iron (II) state. This would eliminate the problem of translocating a tribasic anion through the cytoplasmic

membrane, as the iron (II) species is predicted to possess a net charge of zero. Supportive evidence for this concept is that electrophoresis experiments at pH 3.9 demonstrate that the blue complex is neutral. Furthermore the blue complex dissolves in diethyl-ether.

Because the iron (II)–enterobactin species has a much lower affinity for iron it will readily donate the iron to other coordinating ligands within the cell.

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