Evidence for Mössbauer spectroscopy for different forms of iron core in *Pseudomonas aeruginosa* bacterial ferritin

Nicola M. K. REID,* Dominic P. E. DICKSON,*§ Colin GREENWOOD,† Adrian THOMPSON,† Fahmi H. A. KADIR‡ and Geoffrey R. MOORE‡

*Department of Physics, University of Liverpool, Liverpool L69 3BX, U.K., †School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, U.K., and ‡Centre for Metalloprotein Spectroscopy and Biology, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ, U.K.

Mössbauer spectroscopic studies of whole cells of *Pseudomonas aeruginosa*, grown under different conditions, indicate that the predominant form of iron in the cells varies significantly. These differences are interpreted in terms of differences in the nature of the iron cores of the bacterial ferritin, which result from different growth conditions.

INTRODUCTION

Bacterial ferritins have been isolated from a number of sources: *Azotobacter vinelandii* (Stiefel & Watt, 1979), *Escherichia coli* (Bauminger *et al.*, 1980) and *Pseudomonas aeruginosa* (Moore *et al.*, 1986). These all contain haem groups on some of the protein subunits, and also an iron-rich core, which appears to be somewhat similar to that in mammalian ferritin, hence the designation as bacterial ferritins. The role of these bacterioferritins has not yet been established.

A number of techniques have been used to investigate the form of the iron core in the various members of this group. Electron microscopy has shown electron-dense cores with mean diameters of about 5–6 nm (Moore *et al.*, 1986). High-resolution transmission-electron-microscopy studies have revealed that the cores have a high degree of structural disorder (Mann *et al.*, 1986, 1987), with electron diffraction showing no evidence of crystallinity. The iron contents were generally found to be around 1000 atoms per molecule, which is less than in the case of mammalian ferritins (usually between 1000 and 3000 Fe atoms per molecule). The bacterioferritin cores have a high phosphate content with Fe/P ratios between 1.4:1 and 1.7:1. These data suggest that the bacterioferritin cores are non-crystalline hydrous iron(III) phosphate rather than the ferrihydrite-related structures of other ferritin cores (St. Pierre *et al.*, 1989).

Mössbauer spectroscopy provides a valuable technique for investigating the nature of the iron cores and has been applied to all three examples of bacterioferritin. The Mössbauer spectra of the bacterioferritins from both *E. coli* (Bauminger *et al.*, 1980) and *Ps. aeruginosa* (St. Pierre *et al.*, 1986) show a magnetic-ordering transition at a temperature of about 3 K. This very low value is consistent with a weak magnetic exchange-coupling between the iron atoms, resulting from the low iron density, high phosphorus content and high degree of disorder. However, Mössbauer spectra of the bacterioferritin (Watt *et al.*, 1986) isolated from *A. vinelandii* is very different, with magnetic ordering being observed in the Mössbauer spectrum at much higher temperatures around 20 K. The nature of the transition is also different and corresponds to the superparamagnetic behaviour of magnetically ordered small particles as observed in mammalian ferritins. In the review by St. Pierre *et al.* (1989) it has been suggested that morphological related differences related to the regime under which the iron core was formed can produce

these differences in the Mössbauer spectra despite similarities in composition and degree of order.

An alternative possibility is that the nature of the core is determined by the protein component of bacterioferritin, since it is clear that the isolated bacterioferritins have different physical and chemical properties (J. N. Keen, J. Findlay, F. H. A. Kadir & G. R. Moore, unpublished work).

The purpose of the present work is to investigate whether differences in the iron cores of bacterioferritin can be produced by differences in the growth conditions for the particular bacterium *Ps. aeruginosa*. It has previously been shown (Bauminger *et al.*, 1980) that iron in the same form as bacterioferritin cores represents the overwhelming iron component of whole bacterial cells.

EXPERIMENTAL, RESULTS AND DISCUSSION

Fig. 1 shows the 4.2 K Mössbauer spectra of *Ps. aeruginosa* cells grown under different conditions. Spectrum (a) corresponds to cells grown anaerobically with a high-nitrate medium, and spectrum (b) corresponds to cells grown anaerobically with a low-nitrate medium.

The media are described by Parr *et al.* (1976) and Foote (1986). The carbon sources and many other nutrients were provided by a mixture of yeast extract and peptone. These also provided 10 mg of iron per litre. A further 10 mg of iron per litre in the form of [⁵⁷Fe]ferric nitrilotriacetate was added to increase the proportion of ⁵⁷Fe. A 5-litre batch of cell culture was grown in a Bioflow III fermenter (New Brunswick) with the pH maintained at 6.8 by the addition of 10% (v/v) H₃PO₄. Cell densities were monitored by measurement of the absorption at 600 nm, and cells were harvested during the stationary phase of growth.

It can clearly be seen that in the case of spectrum (a) in Fig. 1 the only spectral component is a single quadrupole-split doublet, which is essentially identical with the spectrum observed from isolated *Ps. aeruginosa* bacterioferritin at this temperature. However, in spectrum (b) in Fig. 1 there are two components, one of which, the central doublet, is very similar to that of spectrum (a), and suggests the presence of bacterioferritin of the type previously observed in *Ps. aeruginosa*. In addition there is

* To whom correspondence should be addressed.

Fig. 1. Mössbauer spectra taken at 4.2 K of intact cells of *Ps. aeruginosa* grown under (a) high-nitrate and (b) low-nitrate conditions

also a strong sextet component with a hyperfine field of approx. 49 T, which is very similar to the spectrum previously observed for *A. vinelandii* bacterioferritin at this temperature.

These results clearly suggest that different forms of iron cores can be produced in bacterioferritins under different conditions of

growth. However, confirmation of this conclusion awaits isolation of the bacterioferritins from cells grown under different conditions.

The support of the Wellcome Trust for the bacterioferritin work at the University of East Anglia is gratefully acknowledged. We also thank the Science and Engineering Research Council for their support at the University of East Anglia Metallobiology Unit via the S.E.R.C. Molecular Recognition Initiative and also for Studentship support of one of us (N. M. K. R.).

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Received 15 August 1990/10 September 1990; accepted 20 September 1990