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E. Bauminger, S. Cohen, D. Dickson, A. Levy, S. Ofer, J. Yariv

To cite this version:


HAL Id: jpa-00218558

https://hal.archives-ouvertes.fr/jpa-00218558

Submitted on 1 Jan 1979

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OBSERVATION OF IRON-STORAGE PROTEIN IN _E. COLI_ BY MOSSBAUER SPECTROSCOPY


Rachah Institute of Physics, The Hebrew University, Jerusalem, Israel
Departement of Biophysics, The Weizmann Institute, Rehovoth, Israel

Abstract. — _57^Fe_ Mossbauer spectra have been measured at temperatures down to 0.08 K in _E. Coli_ cells containing 10 - 1000 _µg_ Fe/1 gram of packed cells. The results indicate the presence of a dominant magnetically ordered iron compound having a transition temperature of 2.6 ± 0.2 K. This provides evidence for the presence of a new type of an iron-storage compound in _E. Coli_. A protein of approximately 500,000 daltons containing an electron dense core, which is probably the magnetically ordered iron compound, has been isolated from the bacteria.

Haem proteins and iron-sulphur proteins have been identified in _E. Coli_. However, no iron storage proteins have been found in this organism or other bacteria. In this study we present evidence based mainly on Mossbauer measurements for the presence of an iron storage protein, containing macroscopic magnetically ordered iron, in _E. Coli_.

In a previous study /1/ Mossbauer spectra were obtained from whole cells of _E. Coli_ grown in _57^Fe_ enriched media. The 82 K spectra consist mainly of an _Fe^{3+}_ doublet with broad lines. The spectra from unfrozen cells at 3°C show large motional broadenings of up to 25mm/s, indicating diffusion of the dominant iron component. In the present work these measurements have been extended to very low temperatures using a pumped _^4^He cryostat and a _^3^He/^He_ dilution refrigerator, in order to gain more information on the nature and relative abundance of the iron compounds in _E. Coli_.

Bacteria were grown and enriched with _57^Fe_ as already described, but with glycerol as the carbon source rather than succinate /1/. The spectra change very little between 82 and 3 K but below the latter temperature the lines begin to broaden and below 2 K there is clear evidence for a six-line magnetically split component (Fig. 1). This component has lines which become broader on going out from the center of the spectrum suggesting that the hyperfine fields at the various iron nuclei are not identical but cover a range of values.

*On leave from the University of Liverpool, England.

**Fig. 1:** _57^Fe_ Mossbauer spectra of a sample of whole cells of _E. Coli_ containing ~ 500 mg/cm³ packed cells with ~ 620 _µg_ _57^Fe_ per gram of bacteria, taken in a range of temperatures. The solid line is a computer fit to the dominant magnetic component using a distribution of hyperfine fields about a mean value.
In addition, there is an Fe$^{3+}$ doublet and an Fe$^{2+}$ doublet which have negligible intensities in samples with high iron concentrations but become appreciable in samples with lower iron concentrations. The spectra were computer fitted to these three components in order to determine their hyperfine parameters (Table I) and relative intensities (Table II).

**Table I**

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Chemical shift (mm/s)</th>
<th>Quadrupole splitting (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$ (Magentically split)</td>
<td>4.1</td>
<td>0.51(1)</td>
</tr>
<tr>
<td>Fe$^{3+}$ (unsplit)</td>
<td>0.08</td>
<td>0.51(3)</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>1.0</td>
<td>0.43(4)</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>1.30(1)</td>
</tr>
</tbody>
</table>

**Table II**

The relative intensities (%) of the components of the 0.1 K Mössbauer spectra of three samples of E. Coli with different levels of iron incorporation (μg $^{57}$Fe per gram of bacteria).

<table>
<thead>
<tr>
<th>Iron incorporation</th>
<th>~ 620</th>
<th>~ 27</th>
<th>~ 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$ (Magentically split)</td>
<td>98 ± 1</td>
<td>58 ± 3</td>
<td>35 ± 6</td>
</tr>
<tr>
<td>Fe$^{3+}$ (unsplit)</td>
<td>&lt; 1.5</td>
<td>15 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>2 ± 1</td>
<td>27 ± 2</td>
<td>29 ± 3</td>
</tr>
</tbody>
</table>

An important feature of these spectra is that the relative intensities of the magnetically split and unsplit parts of the spectra do not vary with temperature, indicating that they arise from different iron compounds. The magnetic component has a mean hyperfine field which varies with temperature (Fig. 2) in a way which corresponds to magnetic ordering with a transition temperature of 2.6 ± 0.2 K and a saturated hyperfine field of 43 ± 1 T. Where there may be some relaxation effects near to the transition the general behaviour cannot be explained by slow paramagnetic relaxation.

It can be seen from Table II that the main effect of varying the iron concentration in the growth medium (and hence the iron incorporation into the bacteria) is to change the relative intensities of the magnetically split and unsplit components. The magnetic component is always present but dominates with samples grown under conditions of iron excess. This strongly suggests that this component is associated with an iron-storage compound.

The observation of magnetic ordering implies that in this compound the iron atoms must be relatively close to each other and grouped in clusters containing a significant number of atoms. However, the magnetically split component is quite different, both in terms of its temperature dependence (no superparamagnetic behaviour), and its hyperfine parameters, from the spectra of the two closely related iron storage proteins, ferritin and haemosiderin, which are found in fungi, plants and higher animals.

The low temperature Mössbauer spectra of E. Coli provides evidence for the presence of an iron-storage compound. Mössbauer measurements carried out on separated fractions from E. Coli show that this compound is in solution within the bacteria.

A protein of large molecular weight (750,000 daltons) characterized by an electron dense core was isolated by us in pure form from an aqueous extract of E. Coli containing 800 mg Fe/l gram packed cells. It seems very probable that this is the iron-storage protein discovered in the Mössbauer measurements. The protein molecule is big enough to suggest that the diffusion broadened spectrum observed by us in E. Coli cells kept above 0°C, which was the topic of a previous report /1/, originates in the aqueous cell interior.

**Acknowledgements.** The Jerusalem group wishes to thank the Stiftung Volkswagenwerk for partial financial support of this work. D.P.E. Dickson gratefully acknowledges financial support from the Lady Davis Fellowship Trust and the Royal Society – Israel Academy Programme. We are indebted to U. Schmid for...
invaluable technical assistance.

References


