State of Iron in the Archaebacterium *Methanosarcina barkeri* Grown on Different Carbon Sources as Studied by Mössbauer Spectroscopy

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Cells of the archaebacterium *Methanosarcina barkeri* were grown strictly anaerobic in defined media. ^{57}Fe-Mössbauer Spectroscopy of methanol grown cells oxidized in the presence of air demonstrated only the state of Fe^{3+} whereas active cells under reducing conditions offered spectra of two additional iron sites in the Fe^{2+} state. Furthermore the ^{57}Fe hyperfine interaction data at different temperatures gave evidence that cells cultivated with methanol or acetate as substrate contained ferredoxin like compounds.

Introduction

M. barkeri is in a certain way an outstanding member of the methanogenic arachaebacteria [1] because it is the only one which can grow on H_2-CO_2 (the common substrate) as well as on methanol and/or acetate as carbon and energy source in a defined medium [2]. Among the methanogens some new coenzymes were found, such as Coenzyme M, Factor 342, Factor 420 and Factor 430, but participants of redox processes typical of other bacteria like menaquinones, ubiquinones and ferredoxins have so far not been isolated or characterized [1, 3]. However, recently membrane-bound cytochrome b_{559} [4] and flavin adenine dinucleotide [5] were found.

As an important spectroscopic tool Mössbauer Spectroscopy could give characteristic information with regard to iron containing compounds [6–8]. In the following Mössbauer spectra of *M. barkeri* are offered which allow a differentiation between H_2-CO_2 and methanol/acetate grown cells and which suggest that in the latter cases the cells contain ferredoxin like compounds.

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Materials and Methods

Culture Methods

Methanosarcina barkeri DSM 804 (strain 'Fusaro') was obtained by Dr. H. Hippe from the Deutsche Sammlung von Mikroorganismen, Göttingen, and cultivated in a defined medium with 5 µM Ni^{2+} (0.5 l vessels) as described previously [9, 10]. Cultivation on methanol was performed in the presence of 25 µM $FeCl_2$ (90% ^{57}Fe) complexed by sodium citrate and reduced by titaniumIII(citrate)$_2$ [2]. Cells grown three and four times on such a medium were used. Cells grown on sodium acetate and H_2-CO_2 had an amount of 0.1 mM naturally occurring $(NH_4)_2$-FeII$(SO_4)_2 \times 6\ H_2O$ as iron source in the medium. *M. barkeri* was more than 20 times subsequently pregrown on sodium acetate (Dr. A. Aivasidis, Jülich) or two times cultivated on H_2-CO_2 as substrate after growth on methanol. The obligate H_2-CO_2 consuming isolate *Methanobacterium thermoautotrophicum* DSM 2133 was obtained from Prof. Dr. R. Thauer, Marburg, and cultivated also in a defined medium, but with 25 µM naturally occurring $FeCl_2$ [11].

Techniques of Mössbauer Measurements

Lyophilized cell material (about 300 mg) was measured in a polyacrylic capsule (about 300 mg/cap-

sule). For reduction wet cells of the same charge were gently thawed up under argon and mixed 1:1 (w/w) with a Na^+-rich anaerobic buffer solution [9] containing additionally 2 mM dithioerythritol and 100 mM methanol. The cells were allowed to reduce themselves by converting methanol to CO_2 and CH_4. After this the cell suspension (0.7 ml) was transferred into the capsule and frozen under liquid nitrogen.

Using a 10 mCi (^{57}Co)Rh-source kept at room temperature the Mössbauer spectra have been measured in the temperature range 1.2–295 K. By means of ring-shaped permanent magnets it was possible to apply a small external magnetic field of 75 G (7.5×10^{-3} T) perpendicular to the γ-ray direction.

Results

As shown in Figure 1 a–h cells of *M. barkeri* grown on methanol or acetate exhibited an obvious Mössbauer spectrum. And as it was in the case of acetate as growth substrate (methanol not shown) a Mössbauer spectrum could also be measured when cells were cultivated in the presence of not enriched Fe (Fig. 1 h). However, cells of *M. barkeri* or *M. thermoautotrophicum* grown on H_2-CO_2 as sole substrate revealed no Mössbauer Resonance. Table I presents the least squares computer fitted hyperfine (hf.) data of the spectra shown in Fig. 1. The spectra of the oxidized cells (Fig. 1 a–d) have been analyzed by a superposition of two quadrupole

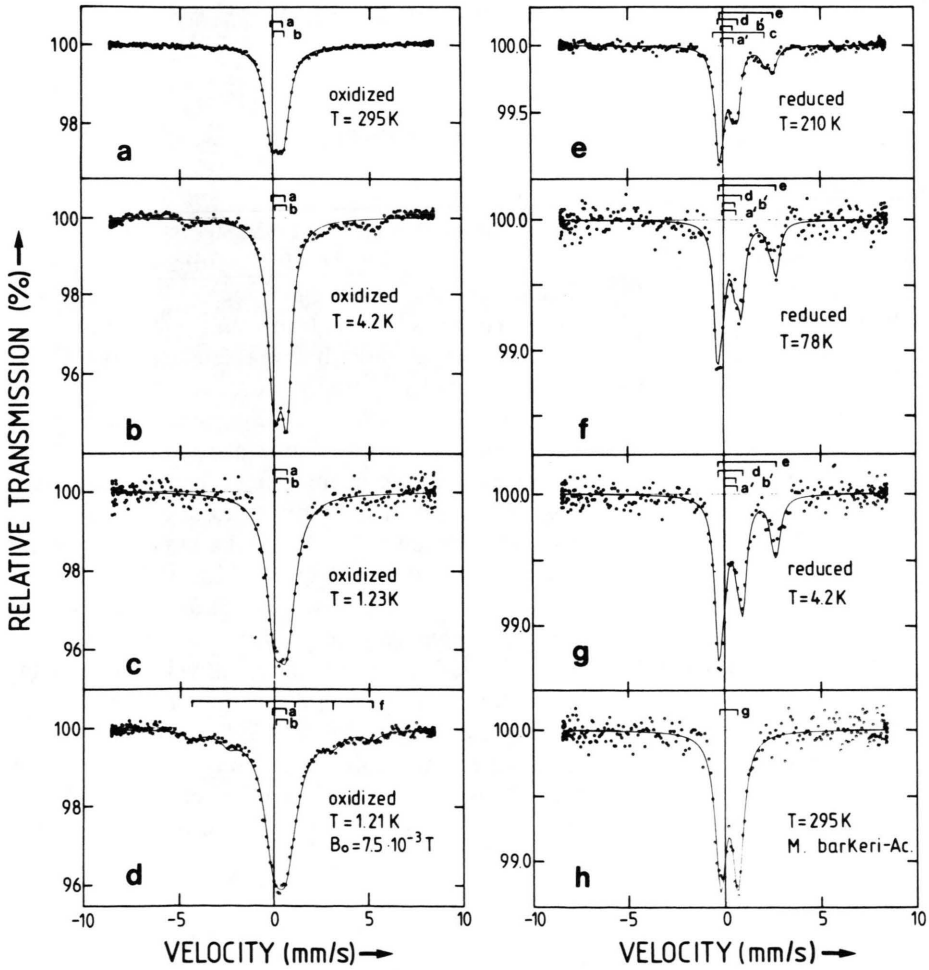

Fig. 1. Mössbauer spectra of oxidized and reduced *M. barkeri* cells grown on methanol or acetate as indicated. For details see text.

Table I. ^{57}Fe hyperfine interaction data of oxidized and reduced *Methanosarcina barkeri* cells at different temperatures pregrown on methanol. The given iron sites refer to the notation of Fig. 1.

Oxidation state	Iron site	Temperature [K]	Isomer shift[a] [mm/s]	Quadrup. splitting [mm/s]	Magnet. hf. field [T]	Linewidth [mm/s]	Area of subspectrum [%]
oxidized	a	295	0.20	0.62		0.74	42
	b		0.31	0.58		0.84	58
	a	4.2	0.31	0.72		0.72	44
	b		0.38	0.61		0.69	56
	a	1.23	0.30	0.69		0.93	46
	b		0.37	0.60		1.15	54
	a	1.21[b]	0.31	0.72		1.23	39
	b		0.39	0.62		1.12	53
	f		0.41	0.08	29.9	0.95	8
reduced	a′	210	0.24	0.64		0.40	10
	c		0.79	2.70		0.46	9
	b′		0.22	0.50		0.45	21
	d		0.26	1.04		0.38	38
	e		1.20	2.82		0.51	22
	a′	78	0.31	0.70		0.45	9
	c		–	–		–	~ 8
	b′		0.28	0.58		0.43	16
	d		0.30	1.28		0.39	39
	e		1.25	3.01		0.44	28
	a′	4.2	0.35	0.74		0.42	6
	c		–	–		–	~ 7
	b′		0.30	0.61		0.49	17
	d		0.31	1.27		0.47	41
	e		1.22	3.04		0.54	29
oxid.-Ac[c]	g	295	0.22	0.89		0.65	100

[a] Isomer shift data refer to a (^{57}Co)Rh-source at room temperature.
[b] Measured in an externally applied magnetic field $B_0 = 7.5 \times 10^{-3}$ T oriented perpendicular with respect to the γ-ray direction.
[c] Cells pregrown on acetate as sole substrate.

splitted subspectra (a) and (b) belonging to ferric iron. The spectrum (Fig. 1 d) measured at 1.21 K and in an external magnetic field of 75 G (7.5×10^{-3} T) clearly reveals in addition a magnetic hf. splitting (f) with a hf. field of 29.9 T (299 KG). Fig. 1 b also may show some indication of a magnetic hf. splitting, but this has not been considered in the fitting procedure.

A contamination of the samples *e.g.* by adhesive FeS sometimes being present in cultures and also by FeII-citrate and $(NH_4)_2$ FeII$(SO_4)_2 \times 6\ H_2O$ could be ruled out by comparison with Mössbauer spectra of these compounds.

The spectra of the reduced cells (Fig. 1 e–g) have been analyzed by two quadrupole splitted subspectra (a′) and (b′) of ferric iron and by two quadrupole splitted subspectra (d) and (e) of ferrous iron. To fit the high temperature spectrum at 210 K (Fig. 1 e) properly an additional quadrupole doublet (c) of ferrous iron proved to be necessary. By cooling down to 1.2 K and applying an external magnetic field of 75 G no indication of any magnetic hf. splitting could be detected with the reduced samples (not shown).

Discussion

The spectra of the oxidized and catabolic active reduced cells reveal obvious but broadened linewidths. This is an indication that the cells could contain more than one iron species. Werber *et al.* investigated another archaebacterium, *i.e.* a halobacterium of the Dead Sea. They also noticed that the spectra of whole cells exhibited broader lines compared with the spectra of the isolated 2Fe-2S fer-

redoxin which was found to be similar to the spinach type [12].

Comparing the spectra with published spectra they show a similar shape like the spectra of 2Fe-2S ferredoxin from spinach measured by Johnson and Hall [13] and Moss *et al.* [14]. In the work of Johnson [13] and Werber *et al.* [12] the iron of the apoferredoxin was not exchanged for enriched ^{57}Fe to avoid any damage of the molecule like it was indirectly done by the technique used here, too. However, the quadrupole splitting of the Fe^{3+}-component of the reduced 2Fe-2S ferredoxin always remains almost independent of temperature [8, 13–16]. Regarding this feature the spectra of the reduced cells of *M. barkeri* cannot be explained any longer by a single 2Fe-2S type compound.

Recently, Huynh *et al.*, and Emptage *et al.* discovered a new 3Fe-centre ferredoxin [17, 18]. Comparing with their data it is possible to interpret the obtained spectra fairly well. Hence, the spectra of the oxidized cells were analyzed by superposition of the two doublets (a) and (b) whereby the Fe^{3+} sites of (a) and (b) can be related to a 2Fe-2S and a 3Fe-3S type compound, respectively. In addition to the doublet (b) the broadened magnetically splitted subspectrum (f) in Fig. 1 d could be attributed to a 3Fe-3S centre, too. This is in agreement with Huynh *et al.* [17], who also found a magnetic hf. splitting of their oxidized sample at 1.5 K and 600 G external manetic field.

The Mösbauer spectra of the catabolic active reduced cell material were analyzed in similar manner by the two quadrupole splitted spectra (a′) and (c) and by the couple (b′) and (d) representing a Fe^{3+} and a Fe^{2+} site of a 2Fe-2S type and a 3Fe-3S type compound, respectively. Thereby the subspectrum (c) should smear out due to relaxation effects at lower temperatures according to [8, 13–16]. To fit the reduced spectra adequately a further quadrupole splitted Fe^{2+}-subspectrum (e) was necessary. Based on a similar finding of Werber *et al.* [12] this subspectrum could be attributed to denaturated protein. However, after this it remains that the lines of the fitted subspectra are still somewhat broader than pure ferredoxins with a linewidth of about 0.35 mm/s.

The presented hyperfine data are not in congruence with haems like cytochrome c (s. literature in 7) or with 4Fe-4S clusters. But recently compounds with a 4Fe-4S or possible a 3Fe-3S cluster, but not with a 2Fe-2S cluster or a haem group were suggested to be present in whole cells of *Methanobacterium bryantii* grown on H_2-CO_2 by using EPR spectroscopy [19].

Ferredoxins so far were not detected in pure methanogenic cultures grown on H_2-CO_2 [3], but from *M. barkeri* cells cultivated with methanol as substrate Blaylock [20] enriched two different ferredoxin fractions which were found to be involved in formation of methylcobalamine from methanol [21]. Therefore further experiments could be promising to prove existence of ferredoxin(s) in *M. barkeri* grown on methanol or acetate.

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