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Co^{2+} interaction with *Azospirillum brasilense* Sp7 cells: a ^{57}Co emission Mössbauer spectroscopic study

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Abstract Preliminary ^{57}Co emission Mössbauer spectroscopic data were obtained for the soil bacterium *Azospirillum brasilense* Sp7 ($T = 80$ K) in frozen $^{57}Co^{2+}$-containing suspensions and in their dried residues. The Mössbauer parameters were compared with those for *A. brasilense* strain Sp245 differing from strain Sp7 by ecological behaviour. Live cells of both strains showed metabolic transformations of $^{57}Co^{2+}$ within an hour. Differences in the parameters observed for the two strains under similar conditions suggest dissimilarities in their metabolic response to Co^{2+}.

Keywords Cobalt(II) metabolism · *Azospirillum brasilense* · ^{57}Co emission Mössbauer spectroscopy

1 Introduction

Cobalt is a trace element with a wide variety of biological functions [1, 2], which at higher concentrations can become toxic [3]. Interaction of bacterial cells with cobalt ions, besides its scientific aspects [2–4], is also of ecological significance [5].

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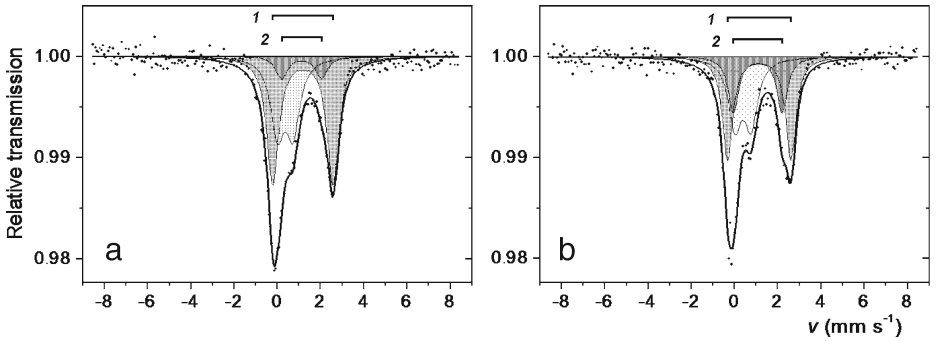

Fig. 1 Emission Mössbauer spectra of *A. brasilense* Sp7 live cells incubated with $^{57}CoCl_2$ (**a**) for 2 min or (**b**) for 1 h and rapidly frozen in liquid nitrogen (measured dried at $T = 80$ K). The positions of the two quadrupole doublets corresponding to daughter $^{57}Fe^{II}$ forms (dark-shaded, 1 and 2; see Table 1, samples "Live (2 min), *D*" and "Live (1 h), *D*") are shown above the spectra (the third light-shaded doublets correspond to daughter $^{57}Fe^{III}$ resulting from after-effects)

The bacterium *Azospirillum brasilense* has long been under study worldwide owing to its phytostimulating potential [6]. This species also represents a good model for studying its responses to ecological factors. Its strain Sp245 is a facultative endophyte (capable of penetrating into and colonising the plant root tissues), while strain Sp7 is an epiphyte (colonising the root surface only). Thus, the two strains of the same species occupy different ecological niches and can show differences in behaviour under similar conditions [4].

Emission (^{57}Co) Mössbauer spectroscopy is a sensitive and informative tool, which has scarcely been used in biology-related studies [2, 4, 7]. In this preliminary work, ^{57}Co emission Mössbauer spectra were measured for live and dead cells of *A. brasilense* strain Sp7 at $T = 80$ K in frozen $^{57}Co^{2+}$-containing aqueous suspensions and for their dried residues. The results are compared with similarly obtained data reported earlier for *A. brasilense* strain Sp245 [4].

2 Experimental

A. brasilense Sp7 cells were grown and prepared for ^{57}Co emission Mössbauer measurements as reported earlier [4]. Emission spectra were measured as in [4] for live and dead cells (hydrothermally treated at 90°C for 1 h) in frozen aqueous suspensions (1.4×10^9 cells ml^{-1}; 0.2 ml, 1.2 mCi $^{57}CoCl_2$, corresponding to 1.2×10^{-5} M $^{57}Co^{2+}$, which is weakly toxic to *A. brasilense* Sp7 [4]) and for their dried residues at $T = 80$ K and processed using the MOSSWINN program [8].

3 Results and discussion

Typical Mössbauer spectra are shown in Fig. 1. The main Mössbauer parameters calculated from the spectra are listed in Table 1. As for strain Sp245 [4], for Sp7 in all cases two quadrupole doublets of daughter high-spin $^{57}Fe^{II}$ components were found corresponding to two chemical forms of parent $^{57}Co^{II}$.

Table 1 Mössbauer parameters calculated from ^{57}Co emission spectra for live or dead cells of *A. brasilense* Sp7 (in brackets, the corresponding data are presented for *A. brasilense* strain Sp245 taken from [4]), incubated with $^{57}CoCl_2$ for specified periods of time and then rapidly frozen in liquid nitrogen (measured at $T = 80$ K), in aqueous suspension (*S*) or dried (*D*)

Samples of bacterial cells	Multiplet[a]	δ,[b] mm s^{-1}	ΔE,[c] mm s^{-1}	A,[d] %
Live (2 min), *S*	Doublet 1	1.10 (1.26)	2.59 (3.00)	56 (44)
	Doublet 2	0.89 (1.20)	2.00 (2.23)	19 (20)
Live (1 h), *S*	Doublet 1	1.16 (1.26)	2.84 (2.89)	35 (51)
	Doublet 2	1.02 (1.16)	2.18 (2.03)	31 (20)
Dead (1 h), *S*	Doublet 1	1.17 (1.24)	2.75 (3.00)	45 (44)
	Doublet 2	1.00 (1.17)	2.13 (2.18)	28 (27)
Live (2 min), *D*	Doublet 1	1.18 (1.24)	2.79 (3.08)	55 (19)
	Doublet 2	1.12 (1.14)	1.84 (2.35)	10 (23)
Live (1 h), *D*	Doublet 1	1.14 (1.22)	2.93 (2.84)	43 (38)
	Doublet 2	1.07 (1.00)	2.25 (2.03)	23 (8)
Dead (1 h), *D*	Doublet 1	1.17	2.78	57
	Doublet 2	1.04	1.95	7

[a]Main doublets corresponding to daughter $^{57}Fe^{II}$ forms stabilised after the $^{57}Co \rightarrow {}^{57}Fe$ nuclear transition (the residual $^{57}Fe^{III}$ forms resulting from after-effects had $\delta \sim 0.36$–0.39 mm s^{-1}, $\Delta E \sim 0.7$–1.0 mm s^{-1})

[b]Isomer shift (relative to α-Fe at room temperature)

[c]Quadrupole splitting

[d]Relative resonant absorption area

Errors: for δ, ± 0.02 mm s^{-1}; for ΔE, ± 0.05 mm s^{-1}; for A, ± 7 rel.%

Dead Sp7 cells, both in suspension (*S*) and dried (*D*; see Table 1), gave very close parameters; slight differences in ΔE and A for their doublets 2 may be attributed to changes in the $^{57}Co^{2+}$ microenvironment caused by drying. In suspensions (*S*), dead cells gave the parameters statistically indistinguishable from those for live cells frozen after 1 h, while for dried samples (*D*) the parameters for dead cells are much closer to those for live cells frozen after 2 min. This can logically be ascribed to possible gradual changes still occurring in the latter while drying.

Note that the contributions from after-effects (see e.g. the third narrow doublets in both spectra in Fig. 1) markedly increased upon drying for live cells frozen 2 min after their contact with $^{57}Co^{2+}$ (from 25% (*S*) to 35% (*D*); see the data in Table 1) and for dead cells (from 27% (*S*) to 36% (*D*)). These contributions, comprised by different yields of after-effects from each ^{57}Co microenvironment, depend on their electron-acceptor properties that may change upon drying which alters the hydration. This may account for changes in the areas of doublets 1 and 2 upon drying. For doublets 1 and 2 (*S* and *D*, 1 h) the δ and ΔE values are close, but their areas change oppositely on drying, assuming different hydration of the Co sites.

For live cells frozen 2 min and 1 h after their contact with $^{57}Co^{2+}$ (measured both as *S* and *D*), notable differences in Mössbauer parameters were found, reflecting metabolic transformations of $^{57}Co^{2+}$ occurring within an hour. (Similar metabolic changes were detected earlier in emission Mössbauer spectra for live cells of strain Sp245 [4].) Also, the increased contributions of doublets 2 in going from 2-min to 1-h samples suggest their relation to ongoing metabolic processes.

However, the Mössbauer parameters for strains Sp245 [4] and Sp7 (see Table 1), obtained under similar conditions, show significant differences both in δ and ΔE for

both quadrupole doublets 1 and 2. This suggests dissimilarities in their metabolic response to Co^{2+}, in line with the data obtained earlier using FTIR spectroscopy [4].

4 Conclusions

Using ^{57}Co emission Mössbauer spectroscopy, live cells of *A. brasilense* strain Sp7 were shown to metabolise $^{57}Co^{2+}$ within an hour, similarly to the data on strain Sp245 of this bacterium reported earlier. Thus, any changes in the Mössbauer parameters observed for live cells can be related to their metabolism, although after-effects have also to be considered.

The Mössbauer parameters for strain Sp7 (in frozen suspensions or dried) differed from those for Sp245 (reported earlier) under similar conditions, suggesting dissimilarities in their metabolic response to Co^{2+}, in line with the previous FTIR spectroscopic data.

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