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Cobalt(II) complexation with small biomolecules as studied by ^{57}Co emission Mössbauer spectroscopy

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ABSTRACT

In the emission (^{57}Co) variant of Mössbauer spectroscopy (EMS), the ^{57}Co radionuclide (with a half-life of 9 months) is used that undergoes a nuclear decay ^{57}Co → ^{57}Fe via electron capture followed by the emission of a γ-quantum, the energy of which is modified by the chemical state and the close coordination environment of the parent ^{57}Co atom. While EMS has been used largely in materials science and nuclear chemistry, its high sensitivity can also be of great advantage in revealing fine structural features and for speciation analysis of biological complexes, whenever the $^{57}Co^{2+}$ cation can be used directly as the coordinating metal or as a substitute for native cobalt or other metal ions. As such EMS applications are yet rare, in order to reliably interpret emission spectra of sophisticated $^{57}Co^{2+}$-doped biosystems, model EMS studies of simple cobalt biocomplexes are necessary. In this work, EMS spectroscopic data are analysed and discussed for $^{57}Co^{2+}$ complexes with a range of small biomolecules of different structures, including 4-*n*-hexylresorcinol, homoserine lactone and a few amino acids (spectra measured in rapidly frozen dilute aqueous solutions or in the dried state at $T = 80$ K). The EMS data obtained are discussed with regard to the available literature data related to the coordination modes of the biocomplexes under study.

1. Introduction

The traditional transmission variant of Mössbauer (nuclear γ-resonance) spectroscopy (TMS), with the stable ^{57}Fe isotope having been most widely used up to now [1–4], has a rich history of applications in a broad range of biology-related and biomedical fields (see, e.g. relevant reviews [5–9] and representative experimental reports [10–14]). For ^{57}Fe TMS, the main methodological limitations are: (*i*) a relatively low abundance of ^{57}Fe in the natural iron (2.19%), so that samples to be studied containing only traces of Fe have to be enriched in ^{57}Fe; (*ii*) a relatively low sensitivity, i.e. with minimal sample requirements of over 1 mM ^{57}Fe and a sample volume about 0.3–0.4 ml [9].

As the Mössbauer effect (i.e., recoilless absorption or emission of γ-quanta) with a noticeable probability can generally be observed in a solid matrix only, solutions or liquid samples are commonly studied in a rapidly frozen state [9,15]. This also allows 'time-resolved' Mössbauer spectra to be obtained (down to a millisecond time scale when using the rapid freeze-quench method [8]), since a rapid freezing of a solution at a certain time point, conserving its structure [15], leads to an abrupt cessation of virtually all ongoing (bio)chemical processes in the system under study. Thus, the subsequent measurement of a Mössbauer spectrum provides a 'snapshot' of the frozen system at the moment of freezing.

In the emission (^{57}Co) variant of Mössbauer spectroscopy (EMS), the ^{57}Co radionuclide (with a half-life of ca. 9 months) is used that undergoes a nuclear decay ^{57}Co → ^{57}Fe via electron capture followed by the emission of a 14.4-keV γ-quantum, the energy of which is modified by the chemical state and the close coordination environment of the parent ^{57}Co atom [1,16,17]. The emitted γ-quanta are then resonantly absorbed in a standard ^{57}Fe-containing absorber. The 'energy scale' is expanded with the help of the Doppler effect (by vibrating the absorber within ca. ±12 mm s^{-1} along the axis *^{57}Co source* (which is the sample in EMS)–*^{57}Fe-containing absorber*; ±1 mm s^{-1} corresponds to ±48.1 neV). This extremely narrow energy interval is nevertheless sufficient to detect any possible instances of γ-resonance, and accumulation of data gives an emission Mössbauer spectrum [16–18].

The EMS variant is significantly more sensitive than TMS and potentially very informative [16]. It requires ca. 10^4-fold lower amounts of ^{57}Co in a sample, as compared with ^{57}Fe TMS, to obtain a good emission spectrum. This is mostly due to the fact that the standard absorber used in EMS studies is normally composed of light elements (e.g., $K_4[^{57}Fe(CN)_6]\cdot 3H_2O$ dispersed in a light polymer), and in biological research the ^{57}Co-doped sample has also very low average atomic

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weight, therefore the electronic absorption of the 14.4 keV radiation is very low. (In standard sources used in TMS the matrix is usually a heavy metal, e.g., rhodium, resulting in a tremendous loss of the 14.4 keV radiation already in the source due to self absorption.) However, owing largely to the evident specific methodological difficulties of this sophisticated nuclear chemistry technique (i.e., the use of radioactive ^{57}Co in the sample under study), the total number of yearly EMS-related publications have tended to diminish from a maximum of about a hundred papers per year (reached in the 1970s of the 20th century) down to ca. 20 papers appearing annually by the 21st century [18].

Despite the evident importance of cobalt as a microelement for biology and biomedicine-related fields and the unique sensitivity of ^{57}Co EMS, its applications in life sciences have so far been featured by quite a limited number of reports. A few studies on vibration characteristics of various ^{57}Co-labelled mammalian ear components were reported in 1964–1974 ([1], pp. 384–386), based on the sensitivity of the Mössbauer resonant system to ångström-range vibration amplitudes [19,20]. Thus, in those earlier studies, ^{57}Co was used merely as an external physical Mössbauer-active label. Several other early and subsequent EMS investigations involved intrinsically biology-related cobalt-containing complexes, such as ^{57}Co-labelled cobalamins (vitamin B_{12} and its analogues), porphyrins and phthalocyanine [21–24]; hemoglobins [25,26] and other heme complexes [27]; the ^{57}Co-doped protein concanavalin A (also used for studying the protein dynamics) [28]; the ^{57}Co-labelled vitamin B_{12} (used in the B_{12}-dependent enzyme system with ethanolamine-ammonia lyase) [23] and, for the first time for an enzyme, in bacterial glutamine synthetase with ^{57}Co-doped active centres [29,30]. Besides that, ^{57}Co EMS was applied for monitoring the state of cobalt(II) in roots of water hyacinth *Eichhornia crassipes* [31] as well as in cells of a cyanobacterium (the blue-green alga *Synechococcus vulcanus*) [32] and Gram-negative bacteria (*Escherichia coli* [33] and different strains of *Azospirillum brasilense* in the freeze-dried state [29] and/or in frozen aqueous suspensions [34,35]).

Owing to the so-called after-effects of the $^{57}Co \rightarrow {}^{57}Fe$ nuclear transformation (see below and [15–18]), which result in line broadening and possible appearance of additional lines in emission Mössbauer spectra, interpreting EMS data for sophisticated biological molecules (such as enzymes) or complicated biosystems may be quite challenging. In such a case, going from the EMS data obtained for simple ^{57}Co biocomplexes to those for complicated biosystems would be very helpful, which justifies EMS studies, accumulation and classification of relevant data on simple model biomolecules, which are yet scarce. In this work, a few examples are considered where EMS is used for studying $^{57}Co^{2+}$ complexation with a range of different relatively small biomolecules. The EMS data obtained are discussed with regard to the available literature data related to the coordination modes of the biocomplexes under study.

2. Materials and methods

For preparing complexes, the following reagent-grade chemicals were used: 4-*n*-hexylresorcinol (also known as 1,3-dihydroxy-4-*n*-hexylbenzene, $C_{12}H_{18}O_2$, purchased from "Sigma") and homoserine lactone hydrobromide (also known as (*S*)-(−)-α-amino-γ-butyrolactone hydrobromide; $C_4H_7NO_2 \cdot HBr$; "Sigma"), chemical analogues of important bacterial autoregulatory substances [36–38]; amino acids – anthranilic (*o*-aminobenzoic) acid ("Serva") and L-aspartic acid (also known as (*S*)-aminobutanedioic acid; linear formula $HO_2CCH_2CH(NH_2)CO_2H$; "Sigma").

Complexes of [^{57}Co]-cobalt(II) were prepared using carrier-free $^{57}CoCl_2$ (i.e., without a natural Co^{2+} salt). For each sample, an aliquot of its aqueous solution (containing 1 mCi of ^{57}Co equal to ca. 2×10^{-9} mol of ^{57}Co [18]) was placed into a polytetrafluoroethylene (PTFE) sample holder, completely dried in air at about 40 °C, and then 1 ml of 10 mM aqueous solution of an amino acid (for L-aspartic acid, 0.2 ml of its 2 mM solution), 4.4 mM homoserine lactone or 7.2 mM 4-*n*-hexylresorcinol was added to the resulting dried remainder (thus giving over two to three orders of magnitude in molar excess of a ligand to $^{57}Co^{2+}$ in each case). After that, each solution was stirred with a glass spatula, closed to prevent evaporation, stored for 1 h to ensure the solution equilibrium and then rapidly frozen in liquid nitrogen prior to EMS measurements, some of which were made in the frozen aqueous solutions and some for air-dried residues made thereof (dried at room temperature and measured at $T = 80$ K).

Emission Mössbauer spectra were collected using a conventional constant-acceleration spectrometer combined with a PC-operated multi-channel analyser and calibrated using α-Fe foil. Samples, which in EMS are sources of γ-radiation, were kept in a specially designed cryostat (with a window for γ-rays) filled with liquid nitrogen (at ca. 80 K). The absorber (containing $K_4[^{57}Fe(CN)_6] \cdot 3H_2O$) was attached to a vibrator vibrating along the direction of the γ-ray. Other details of EMS measurements and statistical treatment of emission spectra were described elsewhere [34,35].

All isomer shift values are presented relative to α-Fe at ambient temperature (for EMS, converted into a form to match the sign convention used in TMS experiments).

3. Results and discussion

Before discussing the EMS data obtained, it has to be emphasized that in ^{57}Co EMS, after the nuclear decay of the parent ^{57}Co cation via electron capture by the nucleus (which thereby turns into ^{57}Fe), an Auger cascade develops within ca. 10^{-14} s [16–18,39–41]. Owing to the Auger-cascade-induced ionisation of the daughter ^{57}Fe ions, which may under certain conditions be not fully reversible by the moment of the emission of a 14.4-keV γ-quantum (ca. 10^{-7} s after the electron capture event), in some cases an additional aliovalent ^{57}Fe charge state can be observed in EMS spectra. This is usually referred to as "after-effects" of the $^{57}Co \rightarrow {}^{57}Fe$ nuclear transformation (e.g. [15], subsection 6.4.1). For instance, such an additional aliovalent state may be represented by stabilised nucleogenic daughter $^{57}Fe^{III}$ species (while the parent compound is represented by $^{57}Co^{II}$ only) which gives its typical spectral components (commonly well distinguishable from those of the nucleogenic $^{57}Fe^{II}$ species). This happens, e.g. if one of the electrons, after leaving the nucleogenic $^{57}Fe^{x+}$ ion (where $x > 2$) in the course of the Auger cascade, gets trapped in the surrounding matrix, finally giving a stabilised $^{57}Fe^{3+}$ ion [15–18].

Such after-effects, though inevitably complicating the EMS spectra, yet can provide additional information on the electron acceptor (and other) properties of the ^{57}Co microenvironment, which has been widely used in radiochemical studies [16,39–41]. Nevertheless, by the moment when a 14.4-keV γ-quantum is emitted, commonly a major or substantial part of the resulting ^{57}Fe ions regain the charge of the parent ^{57}Co ion, staying in essentially the same coordination microenvironment (which is most important). Thus, the resulting substance under EMS study (after the nuclear decay at the moment of emission of a γ-quantum) may be regarded as a ^{57}Fe complex in which the nucleogenic ^{57}Fe ion substitutes for the parent ^{57}Co ion (retaining its charge and the geometry of the surrounding donor atoms of the ligands, the latter being virtually unchanged within the time frame of the $^{57}Co \rightarrow {}^{57}Fe$ nuclear decay up to the moment of emission of a γ-quantum). (Since typical molecular vibrational frequencies are around 10^{-12} s^{-1}, the Mössbauer time window, 10^{-7} s, is enough for the nucleogenic ^{57}Fe to accommodate itself in the ligand environment of the parent ^{57}Co. As a result, in the emission Mössbauer spectrum one can see the iron analogue of a cobalt site keeping the valence state but with slightly different ligand sphere structure. Note that while some slight rearrangement of the ligand positions may occur, the coordination number and basic geometry cannot change due to lack of time for diffusion.)

In other words, since the Mössbauer parameters obtained from both ^{57}Fe absorption and ^{57}Co emission spectra are sensitive not only to the chemical state of the cation and the nature of metal–ligand bonds but

also to their symmetry [1–9,15–18], the data obtained in ^{57}Co EMS experiments may be regarded as a 'snapshot' of daughter ^{57}Fe atoms appearing in the coordination microenvironment of the parent ^{57}Co species. Thus, a comparison between ^{57}Co EMS data for a cobalt complex and ^{57}Fe TMS data for an iron complex with the same ligands sometimes can give valuable additional information. In particular, any differences in the Mössbauer parameters in that case would refer to the different chemical structures of the cobalt and iron complexes reflecting the different chemical properties of these metals.

In the emission spectrum of 4-*n*-hexylresorcinol in dilute aqueous solution (incubated with $^{57}Co^{2+}$ and rapidly frozen in liquid nitrogen), a quadrupole doublet of the nucleogenic $^{57}Fe^{II}$ was observed, with a contribution of a doublet with a smaller quadrupole splitting (nucleogenic $^{57}Fe^{III}$) resulting from after-effects (Fig. 1a; the calculated Mössbauer parameters are listed in Table 1). An apparently similar spectrum was obtained for homoserine lactone (Fig. 1b). It has to be emphasized that for ^{57}Co emission Mössbauer spectra, some noticeable broadening of the lines (as compared with those for ^{57}Fe transmission Mössbauer spectra) is quite typical. Thus, the experimentally measured line width values (W) can commonly reach 0.7 to ~1 mm s^{-1} [21,24,26,34,35], which is caused by several physical and chemical factors intrinsic to EMS [26,39–41]. Therefore, the W values obtained in this work can be regarded as quite common for $^{57}Co^{2+}$-coordinating biomolecules (with possible participation of water molecules in the coordination, at least in some cases).

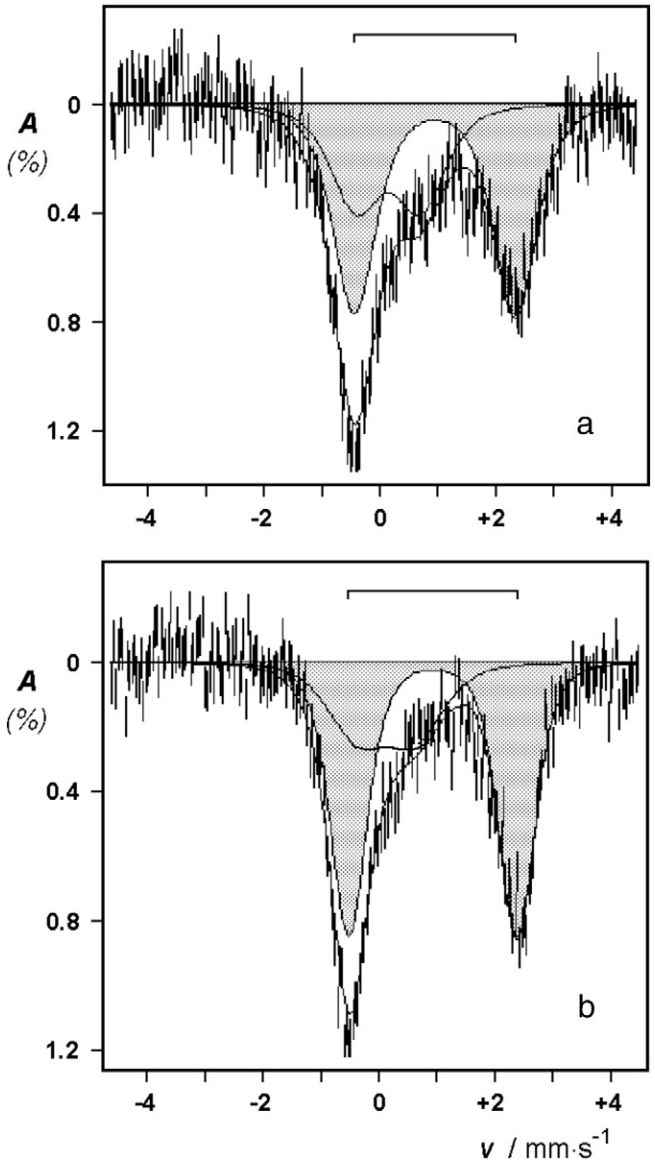

Fig. 1. Emission Mössbauer spectra of (*a*) 4-*n*-hexylresorcinol and (*b*) homoserine lactone incubated with $^{57}CoCl_2$ and then rapidly frozen in liquid nitrogen (measured in frozen aqueous solutions at $T = 80$ K). The positions of the main doublets (shaded areas) corresponding to daughter $^{57}Fe^{II}$ forms (see Table 1) are shown above the spectrum (the other weaker narrow doublets correspond to daughter $^{57}Fe^{III}$ resulting from after-effects of the $^{57}Co \rightarrow {}^{57}Fe$ transition).

Table 1
Mössbauer parameters calculated from ^{57}Co emission Mössbauer spectra for dilute aqueous solutions of biomolecules incubated with $^{57}CoCl_2$ (measured at $T = 80$ K, in frozen aqueous solution or dried).

Biomolecule (state)[a]	Multiplet[b]	δ[c], mm s^{-1}	Δ[d], mm s^{-1}	W[e], mm s^{-1}	S_r[f], %
4-*n*-HR (s)	Doublet	0.91 (5)	2.7 (1)	0.87 (5)	64
4-*n*-HR (d)	Doublet	0.80 (1)	2.48 (3)	0.77 (1)	75
HL (s)	Doublet	0.90 (1)	2.89 (1)	0.78 (2)	71
HL (d)	Doublet	0.8 (1)	2.7 (2)	1.02 (7)	78
AnthrA (s)	Doublet	1.10 (2)	2.71 (5)	0.62 (4)	75
AspA (s)	Doublet 1	1.21 (2)	2.82 (3)	0.60 (3)	72
	Doublet 2	0.96 (7)	2.3 (1)	0.7 (1)	19
AspA (d)	Doublet 1	1.15 (1)	2.60 (1)	0.59 (2)	68
	Doublet 2	0.80 (3)	2.37 (5)	0.63 (7)	24

Calculated errors (in the last digits) are given in brackets.
[a] 4-*n*-HR, 4-*n*-hexylresorcinol; HL, homoserine lactone; AnthrA, anthranilic acid; AspA, L-aspartic acid; emission spectra measured: (s), in frozen solution; (d) in the dried state.
[b] 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, with linewidth (W) values ranging within 0.7–1.0 mm s^{-1}, result from after-effects; their S_r values are 100% minus the corresponding S_r value(s) for the doublet(s) of the corresponding $^{57}Fe^{II}$ form(s)).
[c] Isomer shift (relative to α-Fe at room temperature, converted to the form compatible with conventional transmission Mössbauer spectra).
[d] Quadrupole splitting.
[e] Line width (full line width at half maximum).
[f] Relative resonant absorption area of the quadrupole doublet (in % of the area of the whole spectrum; relative error 7%).

The Mössbauer parameters for the $^{57}Co^{II}$ complexes with 4-*n*-hexylresorcinol (which can coordinate to metal ions via one of its two 1,3-hydroxo moieties; note that their *meta*-position does not allow metal ion chelation by both groups for steric reasons) and with homoserine lactone (which can bind metal ions via both its α-amino and carbonylic oxygen moieties) in dilute frozen solutions are quite similar (see Table 1): isomer shift ~0.9 mm s^{-1} for both complexes; quadrupole splitting 2.7 and ca. 2.9 mm s^{-1}, respectively. This trend is definitely confirmed by our measurements in the dried state (at $T =$ 80 K; see Table 1), which gave the following parameters: isomer shift 0.8 mm s^{-1} for both complexes; quadrupole splitting 2.5 and ca. 2.7 mm s^{-1}, respectively. The slight decrease in the parameters may be ascribed to possible decrease in the hydration state (i.e., in the number of water molecules as ligands which might be replaced by the corresponding biomolecules) upon drying [42,43].

These parameters definitely correspond to high-spin ferrous species; moreover, δ values for high-spin complexes with O and/or N donor atoms equal to or slightly below ca. 1.1 mm s^{-1} (at $T = 80$ K) are common for coordination number 4 which, for cobalt(II), typically corresponds to tetrahedral (T_d) coordination [44]. For comparison, the δ values reported for various high-spin carboxylate-rich Fe^{II} complexes (some with one or two N-donor atoms; measured at $T = 4.2$ K; note that lowering the temperature from 80 K to 4 K could additionally slightly increase the δ) were within the range $(1.04 \div 1.08) \pm 0.02$ mm·s^{-1} for the T_d geometry of the coordination sites (with Δ within ca. $(2.18 \div 3.19) \pm 0.02$ mm·s^{-1}, largely depending on the symmetry) and $\delta = (1.26 \div 1.35) \pm 0.02$ mm·s^{-1} for the octahedral (O_h) geometry, with Δ within ca. $(2.86 \div 3.53) \pm 0.02$ mm·s^{-1} [45]. It has to be noted that tetrahedral coordination is typical for many cobalt(II) complexes with various biological molecules (e.g. [46–48]). In particular, this factor evidently facilitates isostructural substitution of Co^{II} in other tetrahedral sites in bio(macro)molecules, e.g. for Zn [48,49].

The Mössbauer parameters calculated from the emission spectrum for anthranilic (*o*-aminobenzoic) acid (Fig. 2) seem to be also within this trend ($\delta = 1.10$ mm·s^{-1}; $\Delta = 2.71$ mm·s^{-1}; see Table 1). This amino acid is known to chelate metal ions via its carboxylic oxygen atom(s) and its amino nitrogen. In order to ascertain whether the parameters for its $^{57}Co^{II}$ complex in dilute aqueous solution indeed correspond to the T_d symmetry, we can compare them with the parameters and other structural data (obtained by FTIR spectroscopy) for iron(II) anthranilate complex [50]. The FTIR spectroscopic data reported in [50] for solid Fe^{II} anthranilate, particularly those featuring the COO^- vibration modes, definitely evidenced for an O_h coordination of the cation (with two *bidentate* carboxylates and two amino groups as ligands, i.e. a N_2O_4 coordination environment). The ^{57}Fe transmission Mössbauer parameters for Fe^{II} anthranilate (at $T = 80$ K) were reported as follows: $\delta = 1.25 \pm 0.01$ mm·s^{-1}, $\Delta = 2.71 \pm 0.01$ mm·s^{-1}. Thus, the significantly lower isomer shift obtained for $^{57}Co^{II}$ anthranilate complex in dilute aqueous solution (with a large excess of the ligand) evidences for its T_d coordination geometry. In this case, in an aqueous medium, each carboxylic group may be coordinated to cobalt(II) as a monodentate ligand (resulting in a N_2O_2 coordination environment), similar to the $^{57}Co^{II}$-binding carboxylates of amino acid residues in enzyme active centres [30]. It should be mentioned that because of a very low solubility of cobalt(II) anthranilate in water (its mole fraction in its saturated aqueous solution at 25 °C was reported [51] to be 1.26×10^{-7}, corresponding to 7.0×10^{-6} M Co^{2+}), the application of other techniques commonly used to study cobalt(II) coordination in aqueous solutions (e.g., UV–Vis spectroscopy) is virtually impossible. In contrast, EMS is sensitive even to its lower concentrations (1 mCi of ^{57}Co in 1 ml corresponds to ca. 2×10^{-6} M).

It is interesting to note that another amino acid, aspartic (aminobutanedioic) acid, showed a somewhat more complicated Mössbauer emission spectroscopic pattern (a typical spectrum is shown in Fig. 3). Both in dilute aqueous solution (frozen) and in the dried state (at $T = 80$ K) it showed two different subspectra (quadrupole doublets 1 and 2; see Table 1) for high-spin $^{57}Co^{II}$ (together with a relatively small contribution, <10%, from after-effects). The parameters of the minor component of high-spin $^{57}Co^{II}$ (δ, 0.96 and 0.80 mm·s^{-1}; Δ, ca. 2.3 and 2.4 mm·s^{-1} (at $T = 80$ K) for doublets 2 in frozen solution and for the dried residue, respectively) correspond to a T_d coordination geometry. However, the major doublets 1 show

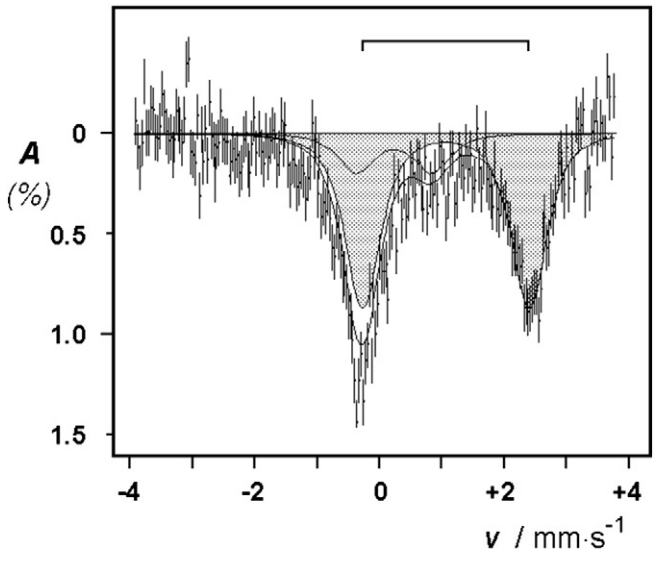

Fig. 2. Emission Mössbauer spectrum of anthranilic acid incubated with $^{57}CoCl_2$ and then rapidly frozen in liquid nitrogen (measured in frozen aqueous solution at $T = 80$ K). The position of the main doublet (shaded area) corresponding to the daughter $^{57}Fe^{II}$ form (see Table 1) is shown above the spectrum (the other weaker narrow doublet corresponds to daughter $^{57}Fe^{III}$ resulting from after-effects of the $^{57}Co \rightarrow {}^{57}Fe$ transition).

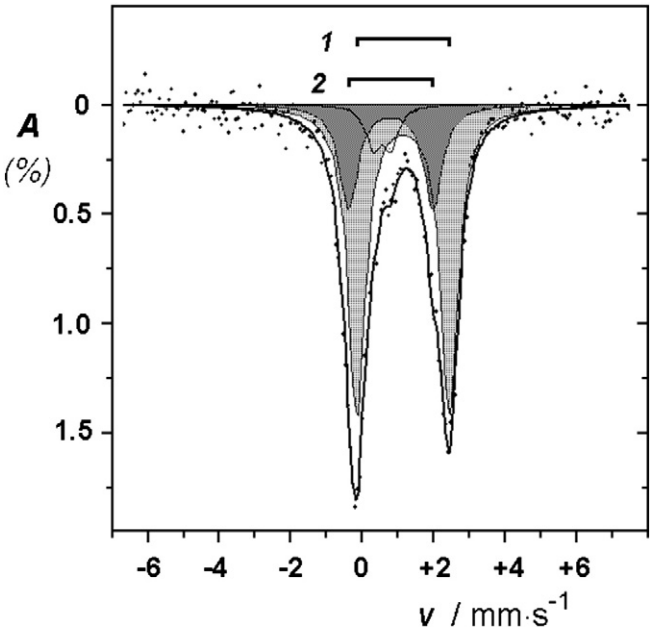

Fig. 3. Emission Mössbauer spectrum of aspartic acid incubated with $^{57}CoCl_2$ and then rapidly frozen in liquid nitrogen (measured at $T = 80$ K in dried solution). The positions of the two main doublets (*1* and *2*; shaded areas of various shading intensity) corresponding to daughter $^{57}Fe^{II}$ forms (see Table 1) are shown above the spectrum (the third weaker narrow doublet corresponds to daughter $^{57}Fe^{III}$ resulting from after-effects of the $^{57}Co \rightarrow {}^{57}Fe$ transition).

the parameters (δ, 1.21 and 1.15 mm·s^{-1}; Δ, ca. 2.82 and 2.60 mm·s^{-1} (at $T = 80$ K) in frozen solution and for the dried residue, respectively) typical for an O_h coordination environment. This dual character of coordination can be attributed to the possibility of aspartate to coordinate metal cations, on the one hand, by one of its two carboxylate moieties together with its adjacent α-amino group and, on the other hand, by its second terminal carboxylic group. It may be reasoned that the former coordination mode (carboxylate plus α-amino group) involving two aspartate anions results in the T_d geometry (with a N_2O_2 coordination environment), while the latter (involving terminal carboxylates only) results in the O_h geometry (with an O_6 coordination environment). This is in line with a higher proportion of the latter component (ca. 70%; see Table 1), which may be a result of a higher binding constant of the complex with negatively charged carboxylates only (as compared with the former complex including also neutral amino ligands) under the equilibrium with a large excess of the ligand. A similar trend has been reported earlier for two different cation-binding sites in the active centres of the enzyme glutamine synthetase, where the site bearing three carboxylates (from three glutamate (Glu) residues, along with possible water molecules) as ligands has a significantly higher affinity for Mn^{2+} [52] and for Co^{2+} [18,30] than the other site, where the ligands include two Glu carboxylates and a neutral histidine residue (with the basic N-donor atom of its heterocycle) [52].

4. Conclusions

^{57}Co emission Mössbauer spectroscopy (EMS) has been proven as an excellent, very sensitive, unique analytical tool for studying the structural chemistry of biologically very important molecules, where minute amount of ^{57}Co-doping and complex formation are available, via the hyperfine interactions measured at the microenvironment of the ^{57}Co local probe. Unique information can be obtained, among other data, on the coordination properties, molecular symmetry as well as on the valence and spin state of the cations.

In the case of Co complexes with 4-*n*-hexylresorcinol and homoserine lactone, the EMS data revealed high-spin divalent state

species with tetrahedral (T_d) coordination of O and O + N donor atoms, respectively. The EMS data for anthranilic acid complex evidenced a T_d coordination symmetry in dilute aqueous solution when each carboxylic group (along with the amino group nitrogen) may be coordinated to cobalt(II) as a monodentate ligand. According to the EMS results, cobalt complexes with aspartic acid showed dual character. One (minor) species was found to be tetrahedrally coordinated, the other one (major) octahedrally coordinated. It is explained by coordination of the α-amino group together with the adjacent carboxylic group of two aspartate molecules to Co^{II} in the tetrahedral complex, while the other terminal carboxylic groups of aspartate anions are involved in coordination in the octahedral complex.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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