Mössbauer, Magnetic and Thermogravimetric Studies of Adriamycin Ferric Complexes

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The 1:1, 2:1 and 3:1 Fe^{3+}:adriamycin lyophylized mixtures have been investigated by means of Mössbauer, magnetic and thermogravimetric methods. The Fe_3Ad complex, in which most likely one ferric ion coordinates to amino sugar and two others are chelated by four oxygens of the adriamycinone ring system, seems to be the major species formed in the studied samples of quelamycin.

Introduction

Adriamycin (Ad), a cytotoxic agent having antibiotic [1] as well as a wide range of anticancer properties [2], reduces its cardiac toxicity when used as the triferric complex [3–6]. The liophylized mixture of three ferric chlorides and adriamycin is called quelamycin. Williams *et al.* [7, 8] recently showed that in diluted aqueous solutions the main species is a 1:1 complex, with iron bound presumably to adriamycinone oxygens.

In this work the Mössbauer and magnetic study of liophyllized quelamycin was undertaken to describe the possible binding modes of iron ions and adriamycin in the solid state.

Experimental

Ferric complexes with adriamycin were synthesized by mixing adriamycin and $FeCl_3 \cdot 6H_2O$ in the appropriate molar ratios in water and by fixing the pH to about 7 [4]. After filtration it was liophyllized and the black-violet powder was used for further studies.

EPR spectra were recorded on a JEOL JES-ME-3X spectrometer at 9.2 GHz at the liquid nitrogen temperature. Magnetic susceptibility was measured by Gouy's method from the liquid nitrogen up to room temperature.

The Mössbauer spectra were measured in a Mössbauer 2330 spectrometer (Polon, Poland). A constant acceleration mode was applied and the results were collected into 256 channels of a multichannel analyzer with 2×10^6 pulse statistics in each channel. $^{57}Co/Cr$ of about 40 mCi was used as a source. The velocity was calibrated by means of $Na_2Fe(CN)_5NO \cdot 2H_2O$ standard (SNP) and the velocity scale zero was assumed to be in the middle of both peaks.

The isomer shift (IS) can be converted into the α-Fe standard by the equation

$$IS_{Fe} = IS_{SNP} - 0.26 \text{ [mm s}^{-1}\text{]}$$

During all measurements the source was kept at 300 K.

The thermal decomposition was made on an OD 102 Derivatograph (MOM Budapest).

Results and Discussion

EPR spectra of all studied samples, *i.e.* those obtained from liophyllization of 3:1, 2:1 and 1:1 Fe(III):adriamycin molar ratio solutions, consist of a broad (~1000 gauss) signal at g = 2.030, characteristic of ferric ion in pseudo-octahedral environment.

Magnetic susceptibility measurements revealed that $1/\chi$ is linear in the studied temperature range (Fig. 1). This linear dependence excludes any considerable exchange interaction between ferric centers *i.e.* there are no oxygen or hydroxyl bridged metal ions in the studied systems [10]. The magnetic moment values can be estimated only roughly since the studied liophyllized samples are the mixtures of the ferric complexes and of NaCl formed during the neutralization of solution with NaOH. Chemical analysis of the studied samples with respect to Fe,

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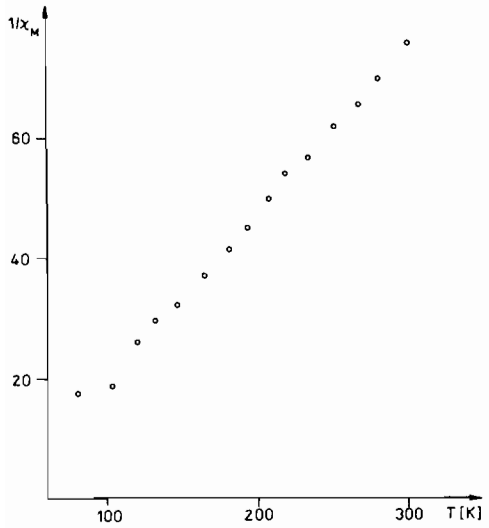

Fig. 1. Temperature dependence of the reciprocal of magnetic susceptibility for Fe_3Ad sample with assumed molar weight 1265 (see text).

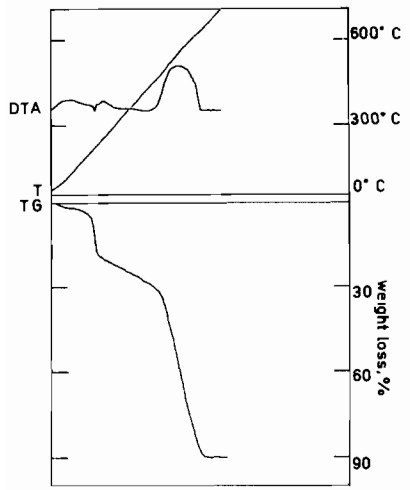

Fig. 2. Thermogravimetric data for metal-free adriamycin. T: temperature scale, TG. weight loss curve, DTA. differential thermal analysis curve.

C, N and H allowed us to approximate the magnetic moment values to 5–5.5 BM. These values may support the EPR conclusion that iron binds adriamycin as a ferric ion. The line broadening measurements of the ^1H NMR spectra of adriamycin when Fe^{3+} ions are added to solution supported earlier findings [4]. Addition of small amounts of ferric ions to the adriamycin solution result in broadening of the NMR resonance lines of ring protons. Higher concentrations of Fe^{+3} influence (among others) the sugar ring proton system, suggesting that adriamycinone ring oxygens as well as sugar donors (NH_2 and/or OH) may be involved in the metal ion binding in the adriamycin complexes with ferric ions. The

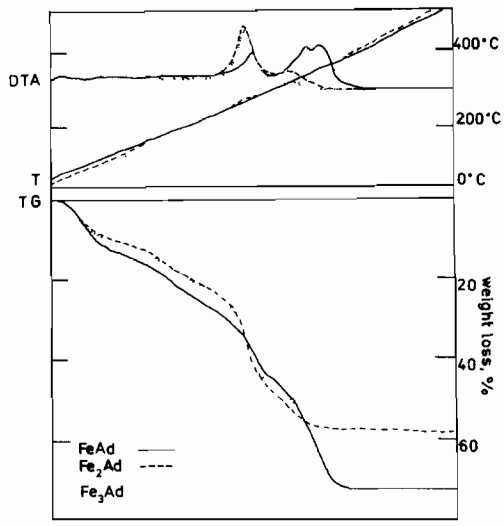

Fig. 3. Thermogravimetric data for the liophyllized samples containing ferric adriamycin complexes. T, TG and DTA as in Fig. 2.

high excess of ligand in the solutions used in this kind of study, however, does not allow the direct correlation between these findings and the results obtained in this work.

The thermal decomposition of the iron adriamycin complexes formed in different molar ratio solutions (Figs. 2, 3) gives useful information about the species created in the liophyllized samples. The metal-free adriamycin undergoes thermal decomposition in two major steps (Fig. 2). At about 200 °C the amino sugar molecule splits its bond with adriamycinone molecule and undergoes thermal decomposition (~25% loss of weight). The thermal decomposition of the remaining part of the ligand molecule proceeds at 450–550 °C.

The thermal decomposition pattern of the samples containing ferric ions and adriamycin is completely different from that for the metal-free ligand (Figs. 2, 3). In the Fe_3Ad species (*i.e.* sample C, which was obtained by liophyllization of the 3:1 Fe^{3+}:adriamycin molar ratio solution) water is removed below 100 °C (~10% loss of weight) and the total thermal decomposition of ligand ceases at 320 °C (Fig. 3). There are no other changes on the DTA (differential thermal analysis) curve up to 700 °C. On the DTA curves of samples A and B (obtained from the 1:1 and 2:1 Fe^{3+}:adriamycin solutions respectively) two or three peaks corresponding to the ligand decomposition can be observed *i.e.* at 260–270, 330 and 350 °C. These results, as well as the results obtained for other molar ratios, strongly suggest that Fe^{3+} ions form three different complexes with adriamycin and it seems to be evident that the Fe_3Ad species characterized by the DTA peak in the 260–270 °C region is formed in all studied solutions. It

TABLE I. Mössbauer Data (Isomer Shift, Quadrupole Splitting and Half Width, Γ) for the Compounds under Investigation.

Compound	T (K)	IS [mm s^{-1}]	QS [mm s^{-1}]	Γ [mm s^{-1}]
FeAd	300	0.62	0.73	0.56
Fe_2Ad	300	0.62	0.73	0.56
Fe_3Ad	300	0.60	0.71	0.54
Fe_3Ad	80	0.72	0.76	0.56
Fe_3Ad^a	300	0.64	0.83	0.64
Fe_3Ad^b	300	0.60	0.73	0.62
		0.48	1.95	
$FeCl_3 \cdot 6H_2O^c$	300	0.80	0.98	

Experimental error of IS, QS and Γ ± 0.02 mm s^{-1}. [a,b]Absorber was measured after ½hour heating of the Fe_3Ad sample at (a) 230 and (b) 340 °C. [c]Detailed description of Mössbauer spectra of $FeCl_3 \cdot 6H_2O$ is given *e.g.* in ref. [9].

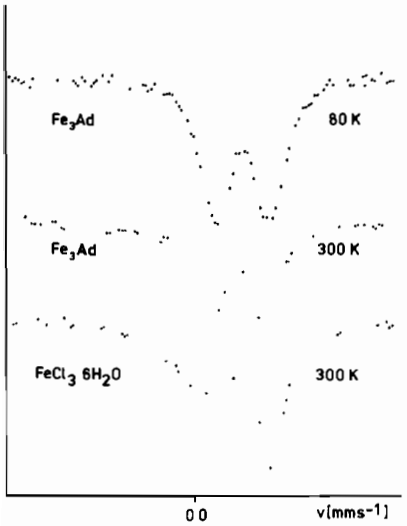

Fig. 4. Mossbauer spectra of Fe_3Ad complex in liquid nitrogen and room temperature. For comparison a spectrum of $FeCl_3 \cdot 6H_2O$ is also presented.

is the predominant complex in the sample (>90%), however, if in the solution used for the liophyllization the Fe^{3+}:ligand molar ratio is close to 3:1. In the other samples the equilibria between FeAd, Fe_2-Ad and Fe_3Ad species are formed. The coordination of metal ion to adriamycin leads in the Fe_3Ad sample to the disappearance of the amino sugar splitting point at 200 °C (see Figs. 2 and 3). These results support the primary NMR line broadening study, which suggested the possible involvement of the amino sugar donors in a ferric ion binding in quelamycin.

The presented results (Figs. 2 and 3), on the other hand, show obviously that the bound metal ion is a strong destabilizing factor which promoted the thermal decomposition of the ligand. Most effective in this respect are three ferric ions bound to adriamycin *i.e.* in the Fe_3Ad complex.

Mossbauer spectra of all studied samples consist of a well resolved symmetric quadrupole doublet. The magnitudes of the isomeric shift (IS) and the quadrupole splitting (QS) are almost the same for all samples studied in this work *i.e.* the samples obtained by liophyllization of 1:1, 2:1 and 3:1 Fe^{3+}:ligand molar ratio solutions (Fig. 4, Table I). The values of IS and QS are characteristic for the high spin Fe^{3+} ion complexes with considerably distorted octahedral symmetry [11, 15].

The Mossbauer spectrum of Fe_3Ad species measured at liquid nitrogen temperature is practically the same as that obtained at room temperature (Table I), except for some small change of isomeric shift due to second order Doppler effect [12]. A constant value of QS is further proof for the high spin Fe^{3+} in complexes formed in the studied solutions.

The comparison of the Mössbauer spectra of $FeCl_3 \cdot 6H_2O$ with those of the studied Fe^{3+} adriamycin complexes (Fig. 4) seems to exclude the presence of the former compound in the liophyllized samples. One well-shaped Mössbauer doublet does not allow us to solve the problem of amino group coordination unequivocally. It seems quite likely, however, that relatively large values of QS obtained for Fe^{3+} adriamycin complexes derive from the fact that besides the oxygen donors, amine nitrogen is also a binding site of Fe^{3+} ion. In other ferric complexes with solely oxygen donors bound to metal ion-like Fe–NTA complexe[13] with heavily distorted octahedral symmetry, the QS value is much lower (0.49 mm s^{-1}) than that observed in our complexes. Even seven-coordinated Fe^{3+} ion in $HFeEDTA \cdot H_2O$ [14] is characterized by a much lower QS value, *i.e.* 0.498 mm s^{-1} than in the adriamycin complexes (Table I). The considerably high line widths (Γ) observed in FeAd species (Table I) are also consistent with unequivalent ferric sites

i.e. different coordination modes of different iron ions.

Following the thermal decomposition of Fe_3Ad complex (DTA curve) we have measured the Mossbauer spectra of this complex after heating at 230 °C (before ligand decomposition – see Fig. 3) and at 340 °C (after major loss of the ligand). The sample heated at 230 °C (loss of most water amount) has larger values of QS in its Mössbauer doublet. It revealed the more distorted symmetry around the ferric ion, which seems to be a logical result of loss of water molecules from the coordination sphere of metal ion. In the Mossbauer spectrum of the samples heated above 300 °C the new doublet appears with very different values of IS and QS as a result of the thermal decomposition of the Fe_3Ad complex (IS = 0.48 and QS = 1.95 mm s^{-1}).

The above results allow us to suggest that iron binds adriamycin as a ferric ion with formation of three different species *i.e.* 1:1 (FeAd), 2:1 (Fe_2Ad) and 3:1 (Fe_3Ad) molar ratio complexes. The binding sites of ferric ions are most likely the four adriamycinone ring oxygens and NH_2 of amino sugar moiety. All formed complexes seem to have considerably distorted octahedral symmetry, with mostly oxygen environment. During the liophyllization process of the 3:1 Fe^{3+}:adriamycin molar ratio solution the Fe_3Ad complex is formed as a major species in the sample. It should be mentioned, however, that according to the recent potentiometric studies [7, 8] in diluted solutions or blood plasma, Fe_3Ad does not exist and the main amount of complexed iron is present as the 1:1 molar ratio species (FeAdOH). It could suggest that quelamycin, when dissolved and diluted or administered to humans, changes its stoichiometry and the ferric ion excess may undergo hydrolysis. This process was observed in aqueous solutions of quelamycin after about ½ hour after dissolution of the complex.

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