COMMENTS ON "THE INTERPRETATION OF THE EPR AND MÖSSBAUER SPECTRA OF TWO-IRON, ONE-ELECTRON IRON-SULFUR PROTEINS" *

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SUMMARY

Evidence is presented indicating that the note mentioned in the title is incorrect in two important aspects. 1.) According to our results of ENDOR spectroscopy and computer simulations of Mössbauer spectra, the conclusions drawn are based on an erroneous interpretation of the Mössbauer spectra. 2.) According to quotations from the literature, previous interpretations of experimental data on iron-sulfur proteins are incorrectly represented.

In a recent note entitled "The Interpretation of the EPR and Mössbauer Spectra of Two-Iron, One-Electron Iron-Sulfur Proteins", Johnson, Cammack, Rao and Hall (1) concluded that EPR and Mössbauer spectroscopic observations support a model of the electron-transferring centers of plant-type iron-sulfur proteins originally advanced by Gibson et al. (2) and Thornley et al. (3), that these proteins contain an iron-sulfur cluster having, on reduction, a ferric and a ferrous atom

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antiferromagnetically coupled to give a complex with a net electron spin of one-half. It is the purpose of this note to draw attention to two aspects of that paper which are incorrect: 1) Johnson et al. (1) have misinterpreted the Mössbauer spectrum of the reduced spinach ferredoxin recorded at 4.2° K. 2) In discussing the results of two of us and their colleagues (4) on the EPR spectra of ^{57}Fe substituted iron-sulfur proteins, Johnson et al.(1) have misrepresented our position while neglecting to point out that their latest interpretation is in direct conflict with their own earlier conclusions (5, 6) on the nature of the active center of the two-iron ferredoxins.

The demonstration by Mössbauer spectroscopy that reduced ferredoxin contains high-spin ferric and ferrous ions coupled antiferromagnetically requires the unambiguous interpretation of the Mössbauer spectra obtained at low temperature with the sample in an applied magnetic field. These spectra are exceedingly complex and synthesized computer fits contain at least 23 physical parameters to characterize the two-iron system. With this many parameters it is difficult to achieve an unambiguous fit and thus arrive at definitive conclusions. It is necessary to combine the results of several different spectroscopic measurements in order to obtain a unique interpretation. Attempting this we have obtained fits (cf. Fig. 1) to our Mössbauer data on parsley and spinach ferredoxins from the results of Mössbauer (7), EPR and ENDOR (8) spectroscopy. These are in direct conflict with the interpretations of Johnson et al. (1), while not contradicting the Gibson-Thornley model. The question must therefore be asked whether proof of that model has been provided by Johnson et al. (1, 9) and whether published data have been correctly interpreted.

The experimental Mössbauer spectra depend on the direction of the applied magnetic field with respect to the γ-ray direction. Our data (7) were taken with H parallel while those reported by Johnson et al. (1, 9) were taken with H perpendicular to the γ-ray direction. The same set of parameters (cf. Table 1) should fit the spectra obtained under both sets of conditions. The computer fit to the data of Johnson et al. using the "best-fit" parameters from our work is

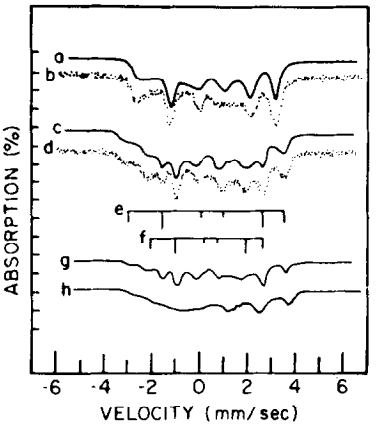

Fig. 1. Synthesized and experimental Mössbauer spectra at 4.2° K and in an applied magnetic field perpendicular to the γ-ray direction. a) The computer synthesized spectrum for an applied field of 0.3 kgauss using the parameters in Table 1, b) the experimental spectrum of Johnson et al.(1) in an applied magnetic field of 0.3 kgauss, c) the computer synthesized spectrum for an applied field of 30 kilogauss using the parameters in Table 1, d) the experimental spectrum of Johnson et al. in an applied magnetic field of 30 kilogauss, e) the so-called "ferrous" stick spectrum of Johnson et al., f) the "ferric" stick spectrum of Johnson et al., g) the ferric portion of the synthesized spectrum in (c), and h) the ferrous portion of the synthesized spectrum in (c).

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given in Fig. 1. These simulated spectra appear to account for all features in the spectra presented by Johnson et al. (1).

Johnson et al. base their arguments on stick spectra which they assign to the ferric and ferrous ions. We include these stick spectra in the figure together with our calculated spectra for the separate ferrous and ferric ion contributions to the total absorption in 30 kG applied field. The anisotropy in the magnetic hyperfine tensor of the ferrous iron results in a comparatively featureless spectrum for this iron atom (see Fig. 1h) which, because of this anisotropy, cannot be approximated by a stick spectrum. On the other hand, the nearly isotropic magnetic hyperfine tensor at the ferric ion gives rise to a sharply detailed spectrum which closely resembles the stick spectra of Johnson et al. Inspection of the figure shows that both sets of stick spectra (Fig. 1e and 1f) in the work of Johnson et al. are due to the ferric atom (Fig. 1g). Apparently Johnson et al. calculated the stick spectrum (Fig. 1e) which they attribute to the ferrous ion using nearly the same isomer shift, quadrupole

TABLE 1

MÖSSBAUER PARAMETERS FOR REDUCED SPINACH FERREDOXIN

	Dunham et al.(7)		Johnson et al.(1)	
	Ferric	Ferrous	Ferric	"Ferrous"
I.S./Pt(mm/S)	- 0.10 ± 0.02	+ 0.19 ± 0.02	-0.13*	+ 0.21*
Q.S. (mm/S)	+ 0.64 ± 0.02	- 3.00 ± 0.1	0.60	0.60**
	0.6 ± 0.03	0 ± 0.2		
A_x' (MHz)	- 51 ± 1	11.1 ± 5.5		
$H_x'^{eff}$ (kG)	- 185	43	-180	180
Ag' (MHz)	-49.1 ± 2.5	16.8 ± 5.5		
$H_y'^{eff}$ (kG)	- 178	64	-180	180
A_z' (MHz)	- 42 ± 1.5	35.3 ± 2		
$H_z'^{eff}$ (kG)	- 172	135	-180	180
g_x	1.89		1.88	
g_y	1.96		1.95	
g_z	2.05		2.04	

Both the effective (S = 1/2) A-tensor components ($A'_{x,y,z}$) and the actual atomic A-tensor components ($A_{x,y,z}$) are expressed in megahertz (1MHz = 3.34 x 10^{-5} cm^{-1}) for the ground (I = 1/2) state of ^{57}Fe.

The equivalent effective magnetic field ($H^{eff} = A\, m_s/g_n\beta_n$) at the nucleus is in kilogauss.

*These isomer shifts have been referred to Pt for comparison.

**This number is deduced by us from the stick spectra of Johnson et al.(1)

splitting and the same magnitude of the effective hyperfine coupling as that used for the ferric ion in Fig. 1f. The only major difference between the two spectra was that the direction of the hyperfine field was reversed; hence the simple (and correct) interpretation of Fig. 1e is that this is the spectrum from ferric atoms in the sample which have the total electronic spin (and hence hyper-

fine field) reversed. Such atoms exist in their sample with a relative Boltzmann probability of 0.3 with respect to the atoms giving spectra as in Fig. 1f. Thus their erroneous conclusion that the effective hyperfine interactions are the same at both iron atoms results from the fact that these authors have only recognized the spectra of the ferric atoms. In this light the only conclusion that can be reached from their treatment of the Mössbauer data is that the total spin of the iron center is one-half. The discrepancy between our results, obtained by Mössbauer computer programs, and the interpretations of Johnson et al. (1), obtained from stick spectra, is apparent from Table 1. The parameters quoted by these authors (1) correspond to the ferric ion only and are incorrect for the ferrous ion according to our results. Furthermore, the interpretation offered by Johnson et al. is in direct contradiction to their own data; e.g., the high temperature data in Fig. 4 of reference 9 show that the quadrupole splitting for the ferrous atom is large as theory predicts, and contrary to their stick spectra. Also, the low-temperature spectra in Fig. 5 of reference 9 show that the lines at 4.2° K occurring at ca. -1.5 and -3.1 mm/sec, and attributed to the ground electronic state of the ferrous atom by these authors, in fact have nearly disappeared in the 1.7° K spectrum, thus indicating that they are from a thermally excited electronic state (ferric, as we indicate) instead. The fact that the lines at positive velocity persist at 1.7° K indicate that the spectrum for the ground electronic state of the ferrous ion has lines at positive velocity (as shown by us in Fig. 1h), but it has no resolved lines at negative velocity, contrary to these authors' stick spectra.

In a paper entitled "The Number of Iron Atoms in the Paramagnetic Center ($g = 1.94$) of Reduced Putidaredoxin", two of us (H. B. and W. H. O-J.) and our colleagues first reported the observation of hfs from two nuclei of spin 1/2 in the EPR spectrum of putidaredoxin (4).† We are now credited

† In subsequent publications on this and related subjects, reference is always made to the more extensive discussion of interpretations and models contained in this paper, viz. ref. 4.

with the interpretation that, in the words of Johnson et al. (1), "In the reduced proteins the iron atoms are identical and therefore the electron is shared between the two." In fact, about the equivalence or identity of the two iron atoms it was stated (emphasis supplied in all instances): "The two iron atoms are not necessarily equivalent as consideration of the model of Gibson et al. (2) and Thornley et al. (3) shows" and "These results do not allow us to choose conclusively among the physical models proposed so far (references to 2, 3, 12) though for this protein at least, complexes involving a single iron atom appear to be eliminated." The thrust of this work in 1967 was to establish the relevance of a two-iron vs. a one-iron model. The "effective" hyperfine splitting observed by us at g_z has been substantiated by ENDOR spectroscopy (8) within limits of error, and in attempts to simulate the line shape at g_z one has to assume that the "effective" hyperfine splitting is within 20% the same for both nuclei and of the magnitude given by us.

It may be recalled in this context that in their original paper Gibson et al. (2) point out that after reduction "the extra electron of the ferrous complex will be in a molecular orbital which will take it onto the ferric complex, reducing the electron affinity of the ferric complex." Gibson et al. (2) also point out that "it is possible that similar models in which an electron is 'shared' between two or more iron atoms are applicable to other ferredoxins." Furthermore, we would like to compare with the above quotations from our work (4) dated March 1968, quotations from papers by Johnson, Elstner, Gibson, Benfield, Evans and Hall from December 1968 (5), and Johnson, Bray, Cammack and Hall of August 1969 (6), both on Mössbauer spectroscopy of iron-sulfur proteins. In 1968 these authors presented the following interpretations: "the magnetic hyperfine interaction seems to have disappeared . . . This could be explained if for some reason the rate at which the electron jumps between one iron atom and the other . . . has slowed down . . To summarize, the low temperature spectra both in zero field and in a small field yield the following information: (1) All the iron atoms seem to give the same Mössbauer spectra, indicating that in the reduced state the single electron must

be equally shared by both irons in the two-iron center of the molecule." Again in 1969 "In conclusion, the Mössbauer spectra show that all the iron atoms in the proteins (two in the ferredoxins studied here . . .) are equivalent in the reduced state, since they give only one spectrum. For these ferredoxins, the reduction is known to be a one-electron process . . . so that the unpaired electron is shared equally between the two iron atoms in the molecule."

We emphasize that we have no fundamental disagreement with the model originally proposed by Gibson, Hall, Thornley, and Whatley (2). Indeed we have obtained a large body of data by EPR, ENDOR, Mössbauer and optical spectroscopy and magnetic susceptibility which provides strong support for this model (7, 8, 13, 14, 15). It is our contention, however, that the conclusions reached in the article by Johnson et al. (1, 9) are based on incorrect interpretations and inferences and do not provide scientific justification for their position. Furthermore, as we have documented in this note, they have incorrectly credited two of us and our colleagues (4) with proposing a certain interpretation (which now appears erroneous) as unique while they have failed to acknowledge the fact that they had proposed this very interpretation themselves.

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