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Mn^{2+}-ELECTRON SPIN RESONANCE SPECTRA OF SEVERAL LECTINS

E. MEIROVITCH, H. BRUMBERGER* and H. LIS
The Weizmann Institute of Science, Rehovot, Israel

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Mn^{2+}-ESR spectra of soybean, wax bean and lima bean agglutinin at Q- and X-band frequencies show nearly axially symmetric zero field splitting (ZFS); the dominant anisotropic term of the spin hamiltonian is the quadratic ZFS interaction. There is a relatively large distribution of ZFS parameters. No effects of specific inhibitor (N-acetylgalactosamine) on the soybean agglutinin spectrum were observed. The stoichiometric complex obtained on addition of Mn^{2+} to a Mn^{2+}-free sample of this protein has a spectrum similar to that of the native protein. The small changes in the spectrum are interpreted in terms of a wider distribution of the ZFS parameters at the Mn binding site. Addition of Ca^{2+} to Mn^{2+}-soybean agglutinin sharpens the lines, possibly because Ca^{2+} increases the rigidity of the complex.

1. Introduction

Metals (in particular manganese and calcium) in lectins have been studied for several years; they appear to be required for agglutinating activity, but their specific role in the protein structure is not entirely clear [1–5]. It is consequently of interest to examine the Mn^{2+} ESR spectra of native soybean agglutinin‡, wax bean agglutinin and lima bean agglutinin(III) [6,7]. We also report here spectra of Mn^{2+}-free SBA to which Mn^{2+} was restored by dialysis against $MnCl_2$ (or, alternatively, $MnCl_2 + CaCl_2$) and of SBA complexed to its specific inhibitor, N-acetylgalactosamine.

2. Experimental methods

SBA was prepared and characterized as described in refs. [8] and [9]. Solutions (6×10^{-5} M) of native SBA were prepared in double-distilled water. The Mn^{2+}-free SBA [3] was prepared by dialysis of native SBA against 1 M acetic acid, followed by extensive dialysis against 0.15 M NaCl and then against distilled water, lyophilization and redissolution in distilled water. This preparation showed no Mn^{2+} ESR signal; Mn^{2+} was restored to it by dialysis against 0.01 M $MnCl_2$ (Mn^{2+}-SBA) [or alternatively, against a mixture of 0.01 M $MnCl_2$ and 0.01 M $CaCl_2$ (Mn^{2+}, Ca^{2+}-SBA)], subsequent dialysis against distilled water, lyophilization, and redissolution in distilled water. Concentrations of the ESR sample solutions ranged from ~ 2.5 to ~ 7 mg/ml. Powder spectra were obtained from lyophilized samples. Solutions were characterized by chromatography on saline Sephadex G-150, and found free of polymers [10]. Agglutination activity was measured by a serial dilution technique [11]; the Mn^{2+}-free SBA was found to have about 40% of the activity of the native SBA, Mn^{2+}-SBA about 60%, and Mn^{2+}, Ca^{2+}-SBA > 80%. Acrylamide gel electrophoresis patterns of Mn^{2+}-free SBA were similar to those of native SBA at pH 4.3 and 8.9, though an additional weak, faster band also appeared at alkaline pH. Mn^{2+}-SBA and Mn^{2+}, Ca^{2+}-SBA also showed comparable patterns to the native protein at both pH regimes. The removal of Mn^{2+} was confirmed by atomic absorption spectroscopy. The Mn^{2+}- and Mn^{2+}, Ca^{2+}-SBA were prepared from aliquots of the same Mn^{2+}-free samples; all of these appeared to retain calcium, in roughly comparable amounts‡.

* Permanent address: Department of Chemistry, Syracuse University, Syracuse, New York, 13210.
‡ Abbreviations: soybean agglutinin – SBA; wax bean agglutinin – WBA; lima bean agglutinin – LBA.
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WBA was prepared and characterized by the method of Sela et al. [12]. LBA in lyophilized form was prepared as described in ref. [3].

ESR measurements were made on a Varian E-12 spectrometer operating at Q-band (TE_{011} cavity) and X-band (TE_{102} cavity) frequencies. For the Q-band measurements, solutions were placed in quartz tubes of 0.1 mm internal diameter; for X-band spectra, solutions were placed in quartz tubes 2–3 mm in internal diameter. All spectra were taken at 23°C.

3. Results and discussion

Solution spectra of SBA and WBA are shown in fig. 1. All these spectra exhibit typical hyperfine structure around $g = 2$. We have not observed signals outside the $g = 2$ region at either X- or Q-band frequencies. Comparison of the signals at X- and Q-band and the measured intensity ratio of the manganese spectrum recorded before and after the Mn^{2+} releasing agent has been added show that the spectra observed in the various Mn^{2+}-protein solutions are due to bound Mn^{2+}. (For the two lectins, this conclusion is supported by the striking similarity of the spectra to that of Mn^{2+}, Ca^{2+}-Concanavalin-A.) Following the method described in ref. [7], we compared the relative total intensities of the observed signals to the intensities of free manganese solutions with the same Mn^{2+} and protein content and in identical sample cells, at X- and Q-bands. In the ESR signal of free

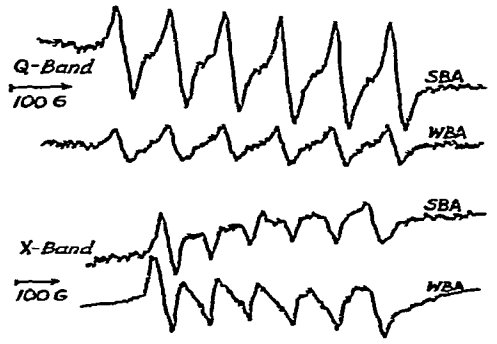

Fig. 1. Q-band and X-band ESR spectra of aqueous solutions of SBA and WBA studied at room temperature. The concentrations were: SBA ~ 6×10^{-5} M, WBA ~ 5×10^{-5} M.

Mn^{2+} all the fine-structure components coalesce into a single sextet. In all cases, the intensity ratio was found, within experimental accuracy, to equal 9/35 = 0.26, which is the relative intensity of the $-1/2 \leftrightarrow 1/2$ fine structure component [6,7]. We therefore conclude that the observed ESR signals are due to this transition only, while the other ($M \neq 1/2$) transitions are smeared out and merged in the background noise.

We have estimated the amount of manganese released from the complex after acidification (by comparing the intensity of the ESR signal arising from such a solution of low pH to that given by solutions of Mn^{2+} aquoions of similar concentration) to be $(2.5 \pm 0.2) \times 10^{-4}$ M. Furthermore, upon adding a small amount of concentrated $MnCl_2$ to a Mn^{2+}-free SBA preparation to obtain a final manganese concentration of 2.5×10^{-4} M, we obtained the spectrum characteristic of bound manganese, identical in shape and intensity with the ESR spectrum recorded after dialysis of demetallized SBA against 0.01 M $MnCl_2$, subsequent dialysis against distilled water, etc. We conclude that four Mn^{2+} atoms bind to one SBA molecule, which at the experimental conditions of the ESR experiment is a tetramer [9].

The ESR behavior described at the beginning of this section is characteristic of powder samples of manganese (^6S) complexes in which the main anisotropic term in the spin hamiltonian is a quadratic ZFS interaction [13]:

$$H = g\beta H \cdot S + AI \cdot S + D[S_z^2 - \tfrac{1}{3}S(S+1)] + E(S_x^2 - S_y^2). \quad (1)$$

When the ZFS parameters D and E are much smaller than the Zeeman term ($g\beta H$), the $M \neq 1/2$ fine structure components will have a first-order dependence on the orientation of the magnetic field and will, therefore, give rise to a widely spread spectrum. On the other hand, the $M = 1/2$ components are to first order stationary in the ZFS parameters and will, therefore, yield relatively narrow lines around $H_0 = h\nu/g\beta$.

The proteins studied in the present work have a molecular weight ranging from 90,000 to 120,000 (table 1). The rotational tumbling times estimated from the Stokes–Einstein equation are too long to average out the spectral spread due to a ZFS interaction of the order of several hundred gauss. We can therefore treat the ESR spectra as powder spectra. Strictly, sharp features are also expected at stationary

Table 1
Magnetic parameters for the Mn^{2+}-protein complexes studied

	M.W. (Daltons)	g	A (gauss)	D (gauss)	ΔH (gauss) a)	
					Q	X
SBA	120,000	2.002 ± 0.001	92	140 ± 30	11	21
WBA	120,000	2.002 ± 0.001	94	160 ± 40	17	27
LBA	90,000	2.002 ± 0.001	94	170 ± 40	16	32
Con A b)	55,000	2.001 ± 0.0005	93	230 ± 10	29	44

a) Average peak-to-peak linewidth of the six hyperfine derivative lines.
b) Mn^{2+}, Ca^{2+}-Concanavalin A [6,7].

field positions of the $M \neq 1/2$ fine structure components. For the hamiltonian in eq. (1) these correspond to resonance fields (nearly) parallel to the principal directions of the ZFS tensor [14–16]. As indicated above, such sharp features were not observed in our protein solutions.

We have studied a number of the Mn^{2+}-protein complexes in their lyophilized powder form. In fig. 2 ESR spectra of such SBA complexes at both X- and Q-band frequencies are shown. The spectra labeled *c* correspond to native SBA (containing both Ca^{2+} and Mn^{2+}), while spectra *a* and *b* correspond to Mn^{2+}-free SBA to which Mn^{2+} and Mn^{2+} + Ca^{2+} were added. Like the solution spectra, these spectra also have typical features of the $-1/2 \leftrightarrow 1/2$ fine-structure component, such as the asymmetric shape of the strong allowed transitions and the weak doublets due to the forbidden transitions. Despite the much better signal-to-noise ratio of these spectra no additional features outside the $g = 2$ region were observed. The lack of such features both in solution and in lyophilized samples is probably due to the distribution in the magnitude of the ZFS. If sufficiently large, this distribution will smear out the sharp $M \neq 1/2$ features beyond detection [17]. The spread in the ZFS parameters may reflect a distribution in the distances between the Mn^{2+} ion and its ligands in the binding sites.

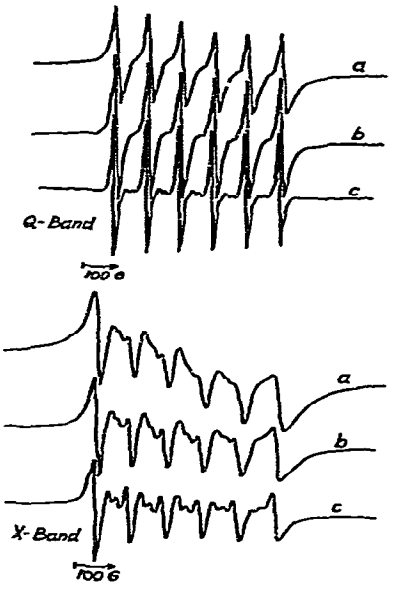

Fig. 2. Spectra of lyophilized powders of (a) Mn^{2+}-SBA, (b) Mn^{2+}, Ca^{2+}-SBA and (c) native SBA at room temperature.

Comparison of the spectrum *a* (Mn^{2+}-SBA) with *b* (Mn^{2+}, Ca^{2+}-SBA) in fig. 2 shows that addition of Ca^{2+} causes some sharpening. Similar effects were observed in con-A complexes and interpreted in terms of increasing rigidity of the Mn^{2+} site upon binding of Ca^{2+}. Addition of N-acetylgalactosamine to SBA did not affect the spectrum either in solution or in lyophilized samples; this was also the case for Mn^{2+}, Ca^{2+}-Concanavalin-A [7].

Thus, based on the ESR observations, we conclude that the various manganese complexes of SBA probably represent the same metal binding site, with various extents of distribution in the ZFS parameters.

The solution spectrum of Mn^{2+} bound to WBA has an appearance very similar to those arising from

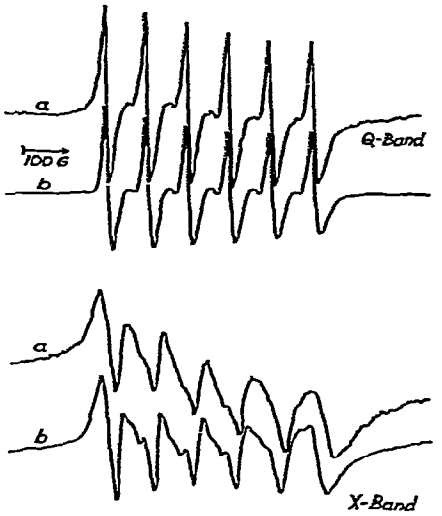

Fig. 3. ESR spectra of native lyophilized powders of (a) LBA and (b) Concanavalin-A at X- and Q-band frequencies.

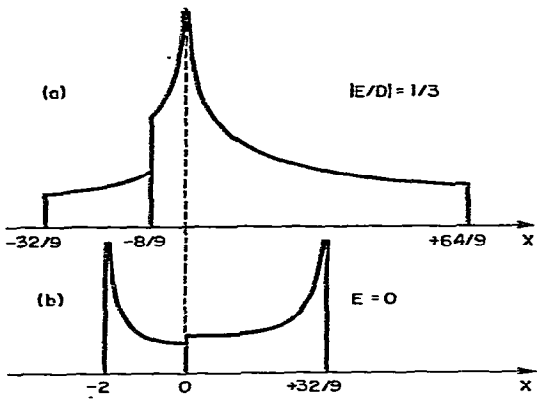

Fig. 4. Second order powder patterns (absorption intensity versus field in units of D^2/H_0) for the $1/2 \longleftrightarrow -1/2$ transition determined by the spin hamiltonian given in eq. (1) ($S = 5/2$ and $D \ll H_0$) (a) for $|E/D| = 1/3$ and (b) for $E = 0$.

the various SBA complexes, the magnetic parameters determining the spin hamiltonian probably being similar.

ESR spectra of lyophilized powders of LBA and Concanavalin-A (fig. 3) are very similar in appearance and frequency dependence; this implies that these proteins have similar magnetic parameters.

We wish to comment in the following on the common characteristic of the ZFS tensors in all the lectins studied and proceed to do so at this stage on the basis of qualitative considerations only.

The spectra of the lectins become lopsided and broaden on going from the Q- to the X-band. We attribute this behavior to the effect of the symmetry of the ZFS hamiltonian. To see this, we consider in more detail the powder spectrum of the $M = 1/2$ fine structure components, which have no first-order dependence on the ZFS parameters. To second order the angular dependence of the resonance frequency for the m'th hyperfine component is [18]:

$$\omega(\tfrac{1}{2}, m) = \omega_0 - mA + \frac{16}{\omega_0}\{(D - E\cos 2\phi)^2 \sin^2\theta \cos^2\theta + E^2 \sin^2 2\phi \sin^2\theta - \tfrac{1}{8}[D\sin^2\theta + E\cos 2\phi(1+\cos^2\theta)]^2 - \tfrac{1}{2}E^2\cos^2\theta \sin^2 2\phi\}, \quad (2)$$

where $\omega_0 = g\beta H_0/\hbar$, and θ and ϕ are the polar and azimuthal angles of the magnetic field relative to the principal directions of the ZFS tensor. The results for two possible extreme values of λ ($= E/D$), i.e., $\lambda = 0$ and $\lambda = 1/3$, are reproduced in fig. 4. It may be seen that in the axial case the overall width of the spectrum, i.e., the separation between the two divergencies, is $(50/9)D^2/\omega_0$. Thus, in this limit the spectral width is expected to decrease with an increase in the applied field, as was observed (table 1). The lack of more detailed structure in the experimental spectra must be due to line broadening, apparently arising from the distribution in the magnitude of the ZFS parameter and to some extent perhaps also to molecular tumbling. From the overall width of the spectra, values for D can be estimated, and are summarized in table 1 together with the isotropic g and A values.

On the other hand, the calculated spectrum for the rhombic case ($\lambda = 1/3$) has a single divergence at ω_0 and is thus stationary, not only with respect to orientation of the magnetic field, but also with respect to the magnitude of the ZFS parameters (as long as $E/D \sim 1/3$). In addition the spectrum exhibits edges which are not stationary with respect to distribution in the ZFS parameters.

While this interpretation of the spectra should be considered with some reservations, it is significant

that in all three agglutinins studied the symmetry of the ZFS is nearly axial.

We are currently performing theoretical simulation experiments of the $1/2 \leftrightarrow -1/2$ transition of the bound Mn^{2+} ion, taking into account distribution in the ZFS parameters as well as molecular tumbling, aimed at a quantitative interpretation of Mn^{2+} complexes with proteins.

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