Vol. 108, No. 2, 1982 **BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS**
September 30, 1982 Pages 673-677

ON THE SPIN AND VALENCE STATE OF IRON IN NATIVE SOYBEAN LIPOXYGENASE-1

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Received August 9, 1982

Summary: Measurements of the magnetic susceptibility of native lipoxygenase-1 yielded a value for the effective Bohr magneton number, n_{eff}, equal to 5.2 which is characteristic for iron in high-spin Fe(II) state.

Upon addition of native lipoxygenase-1 to a butanol-1/D_2O solution a differential line-broadening of the proton resonances in the NMR spectrum of butanol-1 was observed due to relaxation enhancement from interaction between the paramagnetic iron and the protons. This finding excludes the possibility that the iron in native lipoxygenase-1 is in the low-spin Fe(II) state.

These results are consistent with the proposed mechanism of the catalytic function of the iron in lipoxygenase (De Groot, J.J.M.C., Veldink, G.A., Vliegenthart, J.F.G., Boldingh, J., Wever, R. and Van Gelder, B.F. (1975) Biochim. Biophys. Acta 377, 71-79).

INTRODUCTION

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is a dioxygenase containing non-heme iron. The enzyme catalyzes the reaction between molecular oxygen and polyunsaturated fatty acids with a 1,4-*cis,cis*--pentadiene system, like linoleic acid. The products are optically active *cis,trans* conjugated hydroperoxy-dienoic acids [1].

Soybean lipoxygenase-1 (M_r 98500) contains one mol of iron per mol protein. The functional role of the iron has been studied by EPR spectroscopy [2,3]. De Groot et al. [2] have proposed a reaction scheme in which the iron is alternately in Fe(III) and Fe(II) states during catalysis. The native, colourless enzyme is thought to have its iron in the Fe(II) state with oxygen as one of the ligands [2].

0006-291X/82/180673-05$01.00/0

The state of the iron in the yellow-coloured enzyme form obtained by incubation of native enzyme with one molar equivalent of 13-L-hydroperoxy linoleic acid (the main product of the enzymatic dioxygenation of linoleic acid) has been studied by EPR spectroscopy and turned out to be high-spin Fe(III) ($S = \frac{5}{2}$) [2-4]. The native enzyme is virtually EPR-silent and, thus, this spectroscopic method is inadequate for a determination of the spin state of its iron. Mössbauer spectroscopy is not feasible because of serious difficulties with the incorporation of ^{57}Fe. However, measurements of the magnetic susceptibility can be carried out and provide information on the spin state and the valence state of the iron. This will be reported here for the native enzyme.

In an ^{1}H-NMR study [5] a differential line-broadening of the proton resonances of alcohols was observed upon addition of yellow Fe(III) lipoxygenase-1. This line-broadening could be attributed to proton relaxation enhancement from interaction between the paramagnetic iron and the protons of the alcohol. In the present study this method was applied to probe the magnetic properties of the iron in the native enzyme.

MATERIALS AND METHODS

Soybean lipoxygenase-1 was isolated according to Finazzi Agrò et al. [6] with modifications as described by Galpin et al. [7]. The specific activity was 235 µmol $O_2 \cdot min^{-1} \cdot mg^{-1}$, corresponding to 3.92 $µkat \cdot mg^{-1}$. The amount of iron and contaminating manganese were 0.97 and 0.07 mol per mol enzyme, respectively [4]. The amount of high-spin Fe(III) was less than 0.01 mol per mol enzyme as determined by quantitative EPR spectroscopy [4]. Before measurements the enzyme was dialyzed against 0.1 M sodium borate buffer pH 9.0 and concentrated in a Collodion-Bag SM 13200 (Sartorius-Membranfilter GmbH, 34 Göttingen, Germany). The enzyme concentration was determined from the absorbance at 280 nm using $A_{280}^{0.1\%} = 1.6$ with an estimated accuracy of ± 5%.

Magnetic susceptibility measurements were carried out with a sensitive magnetic balance of the Faraday-type as described previously [8,9] except that no sample-deoxygenation was performed. ^{1}H-NMR spectra were recorded on a Bruker 270 MHz NMR spectrometer operating in the Fourier Transform mode. For these experiments the enzyme and alcohol solutions were prepared with 0.1 M boric acid in D_2O adjusted to pH 9.0 with NaOH; the pH meter reading was not corrected for the deuterium effect.

RESULTS AND DISCUSSION

Magnetic susceptibility

Results from the magnetic susceptibility measurements are shown in Fig. 1 where the temperature dependent contribution of the molar susceptibility is given as a function of inverse absolute temperature. Background contributions

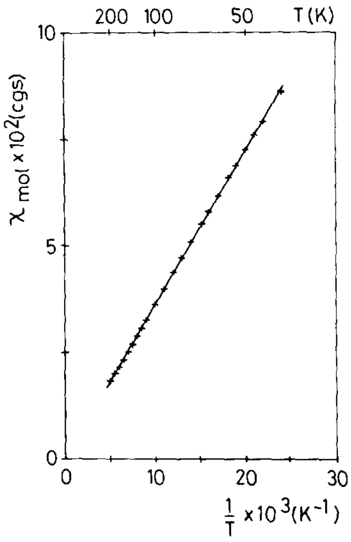

Fig. 1. The temperature dependent contribution of the magnetic susceptibility of native lipoxygenase-1, 103 µl 1.19 mM in 0.1 M boric acid/NaOH buffer pH 9.0.

from the sample suspension wire, sample holder and buffer solution were subtracted. The sample shows Curie dependence of the susceptibility ($\chi \propto 1/T$) in the investigated temperature range of 40-200 K. The straight line (Fig. 1) was fitted to the experimental data by a linear least-square procedure. Its slope, $d(\chi_{mol})/d(1/T)$ was used to calculate the effective Bohr magneton number, n_{eff}, as follows [10]

$$\frac{d(\chi_{mol})}{d(1/T)} = \frac{N\beta^2}{3k} \cdot n^2_{eff}$$

For n_{eff} a value of 5.4 was obtained. This value was corrected for contributions from contaminating manganese (0.07 mol per mol enzyme and probably high-spin Mn(II)) and iron (high-spin Fe(III) [2] and $\leqslant$0.01 mol per mol enzyme) For the corrections the spin-only values for n_{eff} of Mn(II) and Fe(III) were used. The corrected value of n_{eff} (5.2 with an estimated maximal error of ± 0.3) is within the range of the reported values (5.1-5.7) for a system with 6 valence electrons and high-spin configuration (S = 2) [11]. It is significantly different from the reported values of either low-spin (1.7-1.8) or high-spin (5.8-6.0) Fe(III) [11].

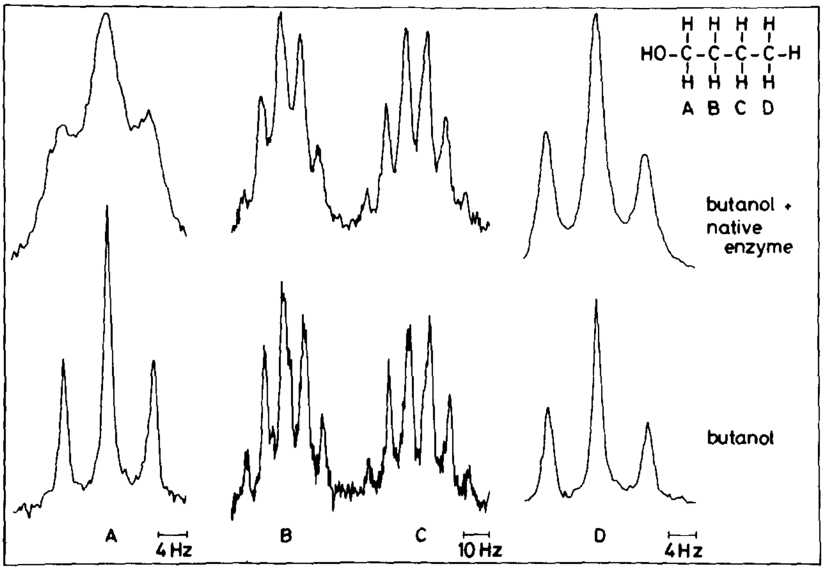

Fig. 2. NMR spectra of butanol-1 showing the effect of paramagnetic iron in native lipoxygenase-1 on the proton resonances of butanol-1. Lower spectrum: 8 mM butanol-1. Upper spectrum: 27 µl native lipoxygenase (0.52 mM) was added to 500 µl butanol-1 (24 mM). Final concentrations: lipoxygenase 26.6 µM and butanol-1 22.8 mM in 0.1 M borate buffer/D_2O pH 9.0. Temperature 297 K.

^{1}H-NMR spectroscopy

^{1}H-NMR spectra of butanol-1 before and after addition of native lipoxygenase-1 are presented in Fig. 2. As also observed for the yellow Fe(III) enzyme the addition of the native enzyme results in a differential line-broadening of the proton resonances of butanol-1 due to interaction between the paramagnetic iron and the protons [5,12]. Since low-spin Fe(II) is diamagnetic this experiment substantiates the conclusion of the magnetic susceptibility measurements that native lipoxygenase has its iron in the high-spin state.

The proton resonances exhibit increasing line-broadening going from the methyl protons to the protons attached to carbon atom 1. This was also observed upon addition of yellow Fe(III) lipoxygenase to a butanol-1/D_2O solution [5]. For the resonances of the methyl protons and the protons bound to carbon atom 1 of butanol-1 the ratio of the observed line-broadening is constant irrespective whether native or yellow lipoxygenase is added. This strongly suggests that the alcohol binds in the environment of the iron of the native enzyme in a similar way as in the yellow Fe(III) enzyme [5]. In contrast to the experiments with yellow Fe(III) lipoxygenase [5], distances between the iron and the protons could not be calculated since information of the electron spin relaxation time of Fe(II) was not available.

It is noteworthy that the optical absorption spectra of native lipoxygenase-1 [13,14] and deoxyhemerythrin which has iron in the high-spin Fe(II) state [15] are almost similar.

In conclusion, from the measurements of the magnetic susceptibility, the ^{1}H-NMR experiments and the optical absorption spectrum it is evident that native lipoxygenase-1 has its iron in the high-spin Fe(II) state. This is consistent with the proposed mechanism of the catalytic function of the enzyme iron in lipoxygenase catalysis [2].

ACKNOWLEDGEMENTS

Thanks are due to Dr. Karl-Erik Falk for assistance with the NMR experiment. Valuable discussions with Drs. Roland Aasa and Tore Vänngård are gratefully acknowledged. We are also indebted to Mr. Lars Mittermaier for skilful technical assistance in the magnetic susceptibility measurements. This work was supported by grants from the Swedish Natural Science Research Council and the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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