limit of 7–8 Å can be set for the separation between the Mn(II) ions in these two sites; beyond this limit, the two ions would have only a small (<10%) broadening effect on one another [4]. This result is consistent with preliminary work of Dunaway-Mariano and Villafranca as quoted in Knight *et al.* [7]. The addition of Ca^{2+} displaces Mn(II) from the partially filled site C and the displaced Mn(II) is redistributed amongst the partially filled sites A and B. Since site C is filled with diamagnetic Ca^{2+}, the Mn(II)–Mn(II) magnetic interaction between sites B and C is suppressed and a narrow line spectrum, corresponding to Mn(II) population of sites A and B, is observed. The increased intensity of this spectrum as a function of added Ca^{2+} is reasonable since the population of Mn(II) at these sites is increased by displaced Mn(II) from site C. Finally, from the observed lack of zero field splitting, the results presented in Figs. 1(b) and 3(d) show that the Mn(II) ions in sites A and B have ligand environments with near octahedral symmetry which are indistinguishable from one another, at least as judged by their EPR spectra.

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Iron Coordination in Soybean Lipoxygenase-1

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A model for the coordination of iron in soybean lipoxygenase-1 is proposed on the basis of an exploratory EXAFS study. Comparison of the EXAFS transmission data of lipoxygenase with those of known metalloproteins, *i.e.* the Cu-EXAFS of superoxide dismutase and the Zn-EXAFS of insulin has led us to suggest that the iron in lipoxygenase is, like the metals in superoxide dismutase and insulin, ligated by histidines coordinating with nitrogen of the imidazole ring. The number of imidazoles is estimated at 3 to 4. EPR spectra of the yellow (ferric) enzyme species indicate an axial symmetry for the iron coordination, making 4 imidazoles likely. A model compound, designed on the basis of this proposal is shown to have considerable lipoxygenase activity.

The oxygen binding of soybean lipoxygenase has been studied by fluorescence, magnetic susceptibility measurements and EPR spectroscopy. The fluorescence of the enzyme is partially quenched upon removal of oxygen by flushing with nitrogen. Magnetic susceptibility measurements show no difference between the air-saturated and de-oxygenated enzyme solutions. This can be explained by assuming that either the oxygen is not coordinated to the iron or it is so firmly attached that it is not removed by the deoxygenation procedure. If the latter were the case, anaerobic addition of 1 equivalent of linoleic acid would result in the formation of product hydroperoxides and in the appearance of an EPR signal of the yellow enzyme species. As this does not appear, oxygen coordination to the iron can be ruled out.

In an earlier EPR study, it was established that the yellow (ferric) enzyme species is reduced by the addition of linoleic acid under anaerobic conditions. Kinetic studies with isotope substitution have shown that the proton abstraction from the methylene group in the 1,4-*cis*,*cis*-pentadiene system of the fatty acid substrate is the rate-limiting step in the dioxygenation catalysis. These studies, in combination with those mentioned above, show that the iron atom is involved only in the activation of the substrate to be oxygenated, as in protocatechuate-3,4-dioxygenase, for example, and not in the activation of the oxygen, as many other oxygenases. Unlike the protocatechuate-3,4-dioxygenase, there is a valence change of iron during the catalytic cycle, the ferric enzyme being reduced in the rate-limiting proton abstraction but reoxidized later in the cycle. As the ferric model compound is also capable of reduction of the fatty acid substrate, the abstracted proton may be localized on one of the coordinating imidazoles and transferred back to the fatty acid later on. The mechanism by which the ferrous native enzyme is activated to the ferric species is still under investigation.