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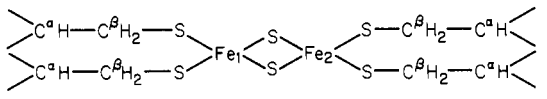
^{1}H NMR Spectra of Reduced Spinach Ferredoxin

Sir:

The ^{1}H NMR spectra of naturally occurring iron–sulfur proteins have been studied for some 15 years by now[1-4] and yet are not fully understood because there is disagreement between the reported spectra and those expected on a theoretical basis. We have investigated the ^{1}H NMR spectra of a reduced 2Fe-2S protein over a quite extended spectral width and detected some more signals that may provide the key for the assignment of the NMR spectra of every kind of iron–sulfur cluster.

Six to eight signals had been detected[1,4-10] in several reduced 2Fe-2S ferredoxins (one iron(III) and one iron(II)) in the range from 45 ppm downfield to 5 ppm upfield from DSS, four of them showing an anti-Curie type temperature dependence and the remaining a Curie type temperature dependence. The first idea was to assign the isotropically shifted signals to β-CH_2's of the four bound cysteines.[1,6] Subsequently, the four signals showing anti-Curie behavior were assigned to the β-CH_2's of the cysteines coordinated to the iron(II) center.[4,5] A theoretical treatment[4] has justified the opposite temperature dependence of the signals related to the iron(II) center with respect to those related to the iron(III) center and provided an estimate of the shift ratios of the protons experiencing the two different metals. The model[4] is based on the assumption that the cysteine protons bound to iron(III) are hyperfine coupled only with this metal ion and, conversely, that protons of cysteines bound to iron(II) are only coupled with the latter ion.



Then, the average $\langle S_{iz} \rangle$ (i = 1, 2) is calculated on all the levels arising from antiferromagnetic coupling, weighted for the different population.[4] It is further assumed that the hyperfine coupling constant is equal for iron(III) and iron(II). Under these circumstances the ratio of the Fe(III)–Fe(II) signals shifts is calculated to be on the order of 10.

We felt that by a careful investigation through NMR equipment with very short dead times between the excitation pulse and the recording of the FID we could gain deeper insight into the problem. Indeed, the spectra recorded on Bruker CXP instruments operating at 300 and 60 MHz revealed the presence of a group of signals of overall intensity 4 above 100 ppm downfield (Figure 1). These signals have a Curie type temperature dependence (Figure 2), as expected for protons close to iron(III).[4] Therefore, we assign these signals to the two β-CH_2's of the cysteines attached to iron(III). There are two more signals above 15 ppm downfield, experiencing isotropic shifts with Curie type temperature dependence. They are assigned to α-CH's of the same cysteines. The difference in shift is somewhat larger than that expected for protons four bonds away from the metal ion. The signal at 42 ppm downfield had already been proposed[5,8] to belong to an α-CH associated with iron(III). Then, there is a set of four signals around 20 ppm downfield whose shifts increase with increasing temperature. They are assigned[4,5] to protons affected by iron(II). Since they have the same shape and similar line widths, they are assigned to the four β-CH_2 protons. The relative positions of β-CH_2's close to iron(III) with respect to β-CH_2's close to iron(II) are of the order of magnitude expected if the shifts were mainly due to contact contributions and the hyperfine coupling were the same for the two ions. Support for this assignment comes from comparison of the 60 and 300 MHz spectra. Signals N, A, and D are sufficiently resolved, even at 60 MHz, to allow comparison of their line widths with those observed at 300 MHz. It appears that signal N, assigned to a pair of β-CH_2's on the iron(III) side, increases in line width on passing from 60 to 300 MHz more than signals A and D; since the increase in line width is mainly due to the onset of Curie spin relaxation mechanisms,[11,12] which depend on $\langle S_z \rangle^2/r^6$, the largest effect is expected for the β-CH_2's bound to iron(III). Other signals are present between 11 ppm downfield and 2 ppm upfield; of these, two (G and K) follow Curie behavior, while I, J, and K are essentially temperature independent. Their assignment is less certain. Poe et al.,[1] besides the four signals with anti-Curie type temperature dependence, found in parsley ferredoxin four additional signals in the 10–30 ppm downfield

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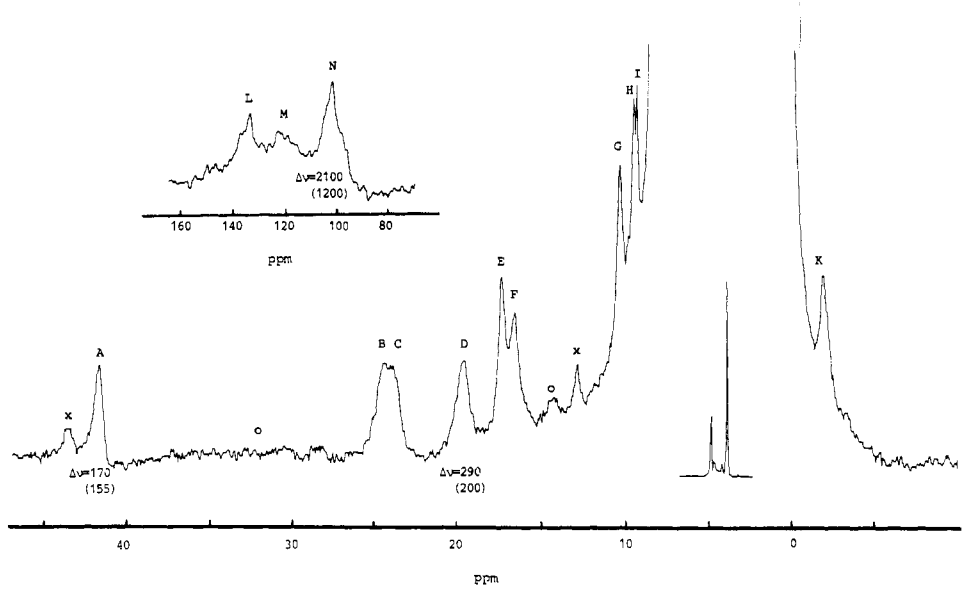

Figure 1. 300-MHz ^{1}H NMR spectrum of reduced spinach ferredoxin in 0.5 M Tris–HCl buffer pH* 7.4 at 24 °C: (O) signals from residual oxidized protein; (×) signals previously observed in spinach ferredoxin solutions and proposed[9] to arise from a recently isolated minor component.[10] The numbers in parentheses refer to the line width of the signals at 60 MHz.

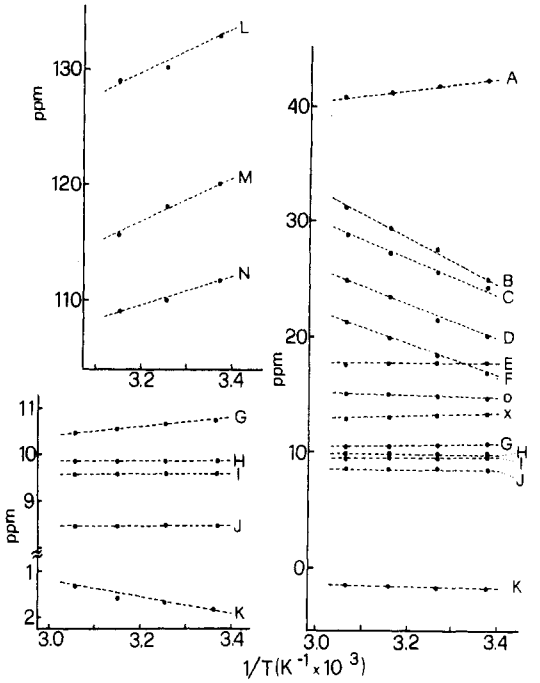

Figure 2. Temperature dependence of the ^{1}H NMR shifts of signals of reduced ferredoxin. The signals are labeled as in Figure 1.

region with Curie or almost Curie type temperature dependence. Nagayama et al.[9] observed only three signals with Curie type temperature dependence in four different ferredoxins; Chan and Markley[8] again observed three such signals downfield and one upfield. It is in our opinion meaningful to note that in the above reports one or two of the signals closest to the diamagnetic region, and thus presumably experiencing less hyperfine coupling have a more pronounced temperature dependence. These signals cannot all be due to cysteine protons near the iron(III) center, since we have detected four more signals above 100 ppm downfield. Therefore, among the three or four signals having Curie type temperature dependence in the region 50 to −5 ppm, only two (the most shifted ones) can be α-CH of iron(III). The other(s) can be assigned to protons of the protein close to the paramagnetic center, thus experiencing a small shift, possibly dipolar in origin. They could also be α-CH's of cysteines associated with iron(II) if dipolar shift contributions are about of the same magnitude as the contact contributions, in such a way as to quench the anti-Curie behavior of the signals. Alternatively, the α-CH's of the iron(II) are in the diamagnetic region.

The wealth of signals observed in the region easily accessible with routine high-resolution NMR spectrometers had previously diverted attention from other signals that were difficult to detect owing to their line widths.

This research shows that a careful search for downfield signals provides evidence for β-CH_2's associated with iron(III) and gives the clue for assigning other signals. Only when similar investigations are performed on a variety of iron sulfur proteins will the relationships between NMR shift pattern and Fe-S cluster type[9,13-16] be put on firm structural basis.

The samples for NMR experiments were prepared by lyophilizing the commercial spinach ferredoxin (Sigma Chemical Co., St. Louis, MO) and redissolving it in D_2O. Reduction of the sample was accomplished by adding solid sodium dithionite in slight molar excess to the protein solution under a dry-nitrogen atmosphere.[1] The NMR spectra were run on Bruker CXP instruments at 60 and 300 MHz on a 100-kHz spectral width, using a modified DEFT[17,18] pulse sequence to allow the suppression of slowly relaxing signals.

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