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A Novel, Stable Bioradical

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Several cellular systems have shown a novel EPR spectrum at 77 K, comprising a slightly anisotropic doublet centred on the free-spin *g*-value, with a hyperfine splitting of *ca.* 120 G. It is suggested that this species is an occluded protein radical centred on a tryptophan unit.

Keywords: Stable bioradical, free radicals, EPR, large proton hyperfine splitting, cell mitochondria

INTRODUCTION

It has recently been found that several proteins contain free radicals which are stable at room temperature and above.[1–5] This surprising result has been explained in terms of weak bonding between the radical and a transition metal complex. However, the crystal structures show that these magnetic units are too far apart for significant stabilisation, and that there will be only very weak magnetic dipolar coupling which contributes nothing to bonding.

In my view, they are "occluded". That means that they are surrounded by inert regions of protein that are so tightly packed that they cannot move laterally. Indeed, the tyrosyl radicals even undergo rotational restriction which is so precise that there is but one orientation of the phenyl group relative to the two β-hydrogen atoms of the RCH_2–PhO unit. Thus, only one gives a hyperfine large coupling. This all fits with the crystal structure of the protein.

I describe such radicals as being "occluded", and that they are very active free radicals, stabilised by immobility. We discovered this phenomenon many years ago for occluded radicals in polyacrylonitrile.[6]

So, if these radical centres are not present to help stabilise the metal centre, what is their purpose? Almost certainly, this is to facilitate electron transfer to such metal centres. Tyrosyl radicals, for example, are powerful electron acceptors, whilst tyrosyl anions are good donors.

These occluded radicals all have their normal EPR spectra. I have now discovered a novel radical occluded in certain proteins, having a 120 G splitting. I call it "the 120 G radical". This has been found in a range of unidentified tissues, but specifically in cell mitochondria, in muscle fibres and in tumour samples. These samples were kindly supplied by the Wellcome Foundation and by Professor Malcolm Jackson.

All samples gave identical EPR spectra (Figure 1). The stick diagram shows that there is

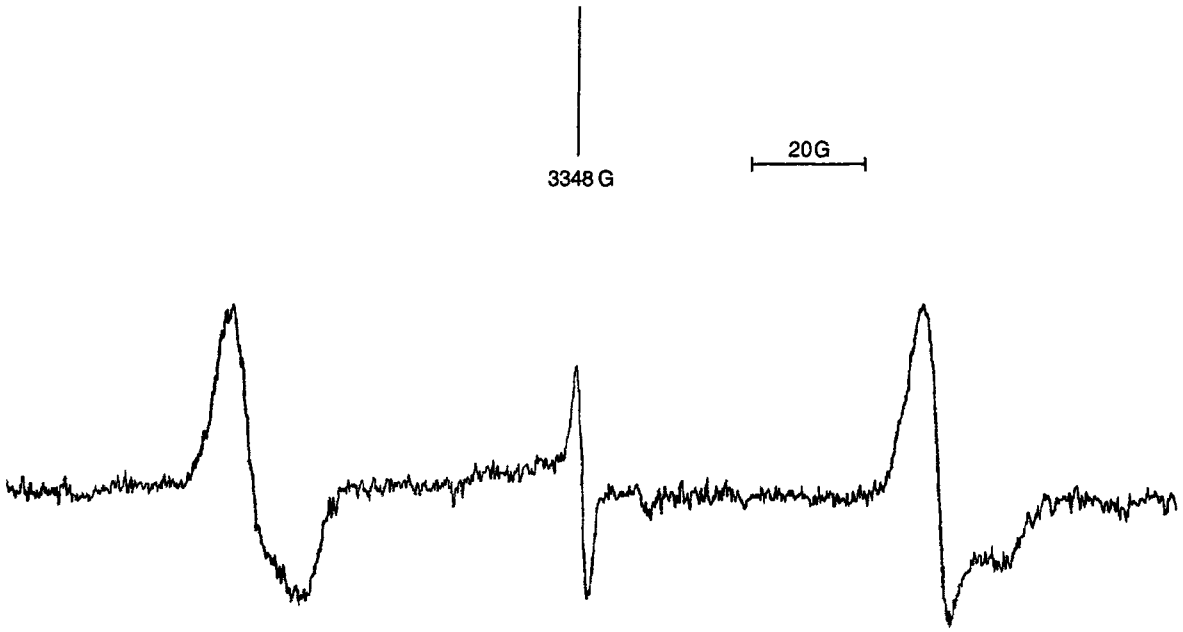


FIGURE 1 First derivative ESR spectrum for the 120 G radical present in mitochondria (This was measured using an X-band Varian E⁻109 spectrometer, at 77 K. Spectra from this and other samples described in the text were identical both at 77 K and at room temperature).

a small hyperfine anisotropy but that the g-tensor is almost isotropic at $g = 2.0025$.

SPECTRAL ANALYSES

The doublet hyperfine data and g-values are given in Table I, together with data for related radicals. All these were identical, the spectra being superimposable. Whilst I favour a simple doublet, there are at least three alternatives. One is that there are two separate iron complexes, one with three high g-values, and one with three low g-values. However, Q-band EPR studies gave exactly the same spectrum showing that the g-values are isotropic.

The second is that there is a central line from a triplet. For several samples, no central line was observed (Figure 1) ruling this out. A third possibility is that it is a spin–spin multiplet involving two proximal radicals. Again there are too few features for this possibility.

Next, I consider the isostructural radicals $\dot{H}CO$ and $\dot{H}CN^-$, which are amongst the very few that have such large proton splittings. It is interesting that several radiation experts have detected $\dot{H}CO$ radicals in irradiated DNA and its components.[7,8] Van de Vorst and co-workers replaced the H_2O matrix with D_2O and detected $\dot{D}CO$ thus confirming the identification.

There are problems concerning the possible source of this radical. They propose that it comes from the sugar units of the DNA. However, only methanol, of the alcohols, can give $\dot{H}CO$.[9]

$$H_3COH \rightarrow H_2\dot{C}OH \rightarrow H_2CO \rightarrow \dot{H}CO$$

It is difficult to formulate any way for obtaining $\dot{H}CO$ from the sugar units, and none was offered. Hütterman and co-workers suggested that the radicals might alternatively stem from the phosphate units with a large ^{31}P hyperfine splitting, but the $\dot{D}CO$ result rules this out. I propose that the $\dot{H}CO$ radical forms from the common impurity CH_3OH, or possibly H_2CO. Certainly we have never detected this centre in our studies of DNA.[11]

TABLE I ESR parameters for the 120 G centre in a range of samples and for some related radicals

Radical	^{1}H hyperfine coupling (G)[a]			g-values		
	$A_{\parallel}$	$A_{\perp}$	A_{iso}	$g_{\parallel}$	$g_{\perp}$	g_{iso}
HCO[b]	133	136	134.5	2.000	2.002	2.0013
120 G centre[c]	119.5	120	119.8	1.996	2.0045	2.0017
$H\dot{C}N^-$[d]	137	137	137	1.997	2.004	2.0017

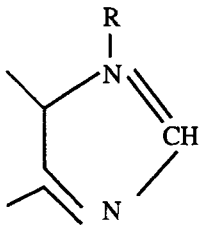
[a]G = 10^{-3} T; [b]Lanolt–Bornstein, Vol. 11-9a, Springer-Verlag, Berlin, 1977; [c]this work; [d]Ref. [10].

Also, this centre was lost rapidly after warming above 77 K whereas the present centre is stable well above room temperature. Hence, it cannot be a strange form of $H\dot{C}O$. The HCN^- radical is even less probable since it protonates in water to give $H_2C\dot{N}$.[10]

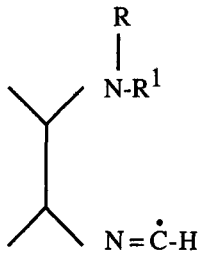
POSSIBLE STRUCTURES FOR THE 120 G SPECIES

I consider proteins first since these are so good at giving occluded radicals. There are two prime candidates, guanine and cytosine.

For example, I consider the unit



This can react with a radical $R^{\bullet}$/ to give



So far as I can tell, this novel radical may well be responsible for the large proton splitting.

Radicals of this type can also be formed in a similar way from guanine and cytidine. However, although it is clear that such a radical formed in a suitable part of a protein may well be occluded, and hence stabilised at body temperatures, this too seems less probable for DNA. However, for DNA in tightly packed chromatin it may be possible. In our own studies, using aqueous DNA, chromosomes and cell nuclei, we found that radicals generated at 77 K were totally lost in the region of 150 K. The absence of resolved ^{14}N hyperfine coupling is not surprising, since it is expected to be small. From the line-widths it must be less than *ca.* 2 G.

I therefore stronly favour the radical (I) formed from histidine for the 120 G species.

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