Radiation Processes in Red Blood Cells: E.s.r. Evidence for Electron Loss by Iron(II) Haemoglobin

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Summary Red blood cell suspensions, after exposure to ^{60}Co γ-rays, gave e.s.r. features characteristic of Fe^{III} in a spin-paired configuration.

Although radiation effects in a wide range of biological materials and model systems have been studied by e.s.r. spectroscopy,[1] there are no studies in which the iron atoms of haemoglobin or other iron porphyrin systems have been directly implicated. When aqueous red blood cell suspensions, rapidly frozen to 77 K, were exposed to ^{60}Co γ-rays, a low-field e.s.r. signal exhibiting four distinct features was obtained (Figure 1a). Spectra at Q-band frequencies confirmed that these are all g-features [$g_x + g_y$ for two distinct centres, α and β] and also revealed two high-field features which constitute the third set for the two centres (g_z for α and β).

Since no known sulphur radicals have features of this type,[2] we conclude that both centres, which must be very similar (Table), are formed by loss of an electron from Fe^{II} presumably in haemoglobin. This is supported by the appearance of a feature in the $g = 6$ region, characteristic of normal, spin-free, Fe^{III} after warming to near the softening point (Figure 1b). This occurred with loss of species α and β, but a third low-spin species (γ) was formed simultaneously (Table). Pure haemoglobin gave the same centres when irradiated, but the cell membranes alone gave no low-field features, which again supports the suggestion that it is the haeme iron atom that is responsible.

The central lines (not shown in Figure 1) contain features characteristic of hydroxyl radicals,[3] and of the well-known thymyl radicals.[4] The hydroxyl radical signal annealed rapidly, but no growth of any signal assignable to Fe-con-

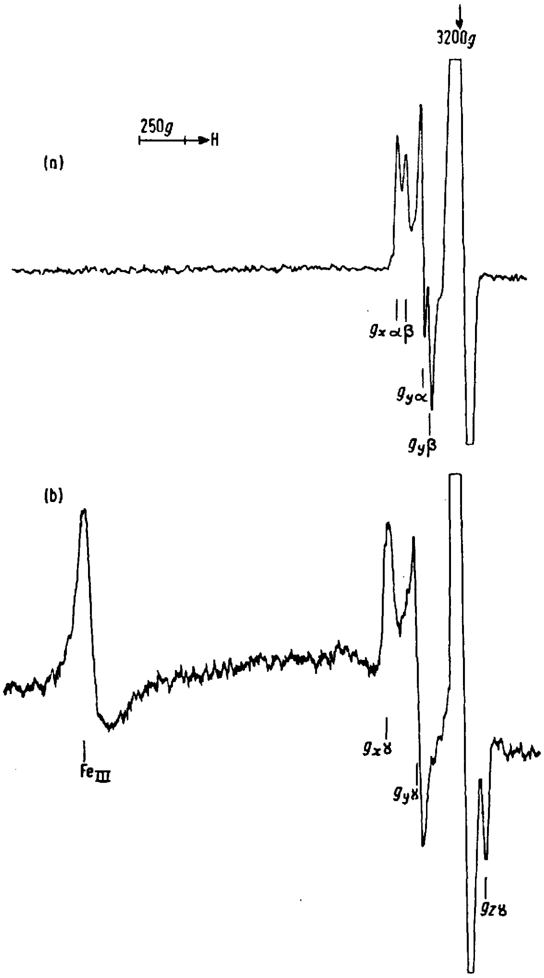

FIGURE. First derivative X-band e.s.r. spectra for aqueous red blood cell suspensions after exposure to ^{60}Co γ-rays at 77 K. (a) At 77 K, showing features assigned to centres α and β, (b) After annealing and recooling to 77 K, showing the γ-centre together with the $g = 6$ feature for normal spin-free $\mathrm{Fe^{III}}$.

taining centres was observed. This suggests that the $\mathrm{Fe^{III}}$ centres α and β are not formed by the addition of ·OH to $\mathrm{Fe^{II}}$.

Many low-spin ferric haeme compounds are known,[5] all being characterised by three g-features. Two examples are included in the Table. However, none prepared by chemical means have the parameters assigned to α or β centres, which exhibit a smaller spread of g-values than any of the others. These low-spin complexes are generally thought to have a configuration approximating to d^2_{xy}, d^2_{yz}, d^1_{xz} for the five electrons on iron,[5] (where z is normal to the porphyrin plane, and x and y are close to the Fe-N directions). The d_{xz} and d_{yz} orbitals are mixed by H_z and this causes the large positive shift for g_z. Similarly, coupling between d_{xz} and d_{yz} causes the smaller shift for g_y, to a first approximation. It seems probable that the $\mathrm{Fe^{III}}$ is initially formed with the ligand environment of the parent $\mathrm{Fe^{II}}$. [The two forms, α and β, which are always present in almost equal amounts, may well be formed from the α and β units in the haemoglobin groups]. On annealing, the ligands can relax to more favourable sites, leading either to species γ, or to spin-free $\mathrm{Fe^{III}}$.

E.s.r. parameters for centres α, β and γ formed in irradiated red blood suspensions

	g-values			
Centre	g_z	g_y	g_x	g_{av}
α	2·233	2·127	1·958	2·106
β	2·205	2·107	1·967	2·093
γ	2·285	2·159	1·940	2·128
$\mathrm{Fe^{III}}$-OH[a]	2·55	2·17	1·85	2·19
$\mathrm{Fe^{III}}$-py[b]	2·41	2·26	1·93	2·20

[a] o-type in ref. 5 (hydroxy haemoglobin).
[b] p-Type in ref. 5 (pyridine haemoglobin).

The very high yield of these centres relative to others should be noted. The normal 'hole' centre in irradiated aqueous glasses is ·OH, even in the presence of other substrates. Thus these experiments reveal a remarkable ability of the iron atoms to scavenge primary holes prior to permanent trapping. [The results for centres α and β were the same when sufficient glucose was added to ensure glassification of the suspensions when cooling].

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