EPR and ENDOR studies of ferrous hemoproteins radiolytically reduced and oxidized at 77K. Evidence for conformational substates in pentacoordinate ferrous hemoproteins

Roman Davydov, Brian M. Hoffman
Department of Chemistry, Northwestern University, US

Exposure of frozen solutions of reduced hemoproteins where heme iron (II) is in the pentacoordinate high-spin state (ferrous myogobin, α– and β-chains of human hemoglobin, carp hemoglobin , horseradish peroxidase) to γ-irradiation at 77K yields EPR active reduced (Fe(I), $3d^7$) and oxidized (Fe(III), $3d^5$) heme centers which retain the conformation of the ferrous precursors. The cryoreduced species show EPR and ENDOR spectra (Fig.1) similar to these seen previously for low-spin (S=1/2) pentacoordinate Co(II) and Fe(I) porphyrins.[1] Interestingly, hexacoordinate low spin Fe(II) porphyrin derivatives forms only Fe(III) and not Fe(I) states during cryoradiolysis. EPR spectra of cryoreduced myoglobin, α– chain and carp hemoglobin desclose two distinct EPR signals with different relaxation properties.(Fig.1) This finding indicates that the deoxy precursors adopt different conformational substates. The Fe(I) species decay at temperatures above 200K.

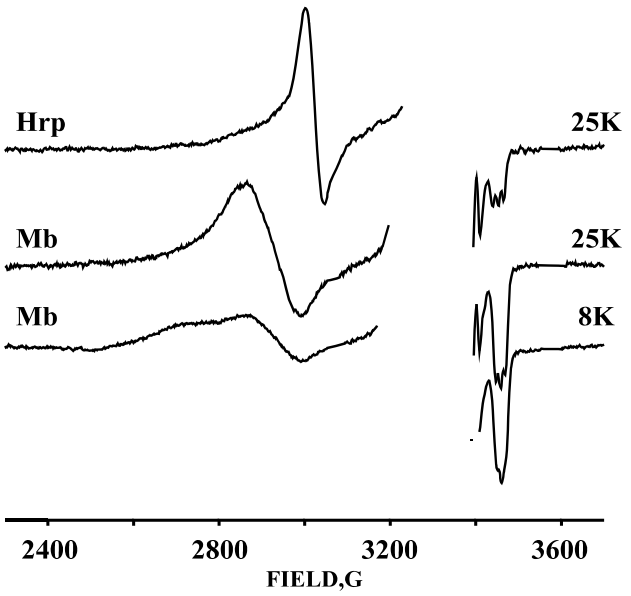

Fig.1 EPR spectra of cryoreduced ferrous peroxidase and myoglobin

Radiolytic cryooxidation of the ferrous hemoproteins generates pentacoordinate high-spin ferric states which, as rule differ, from those of the relaxed hexacoordinate high-spin aquo-ferrihemes that form during annealing at T>200K.

[1] Donohoe R.J. et al. J.Am.Chem.Soc. (1987), **109**, 5593.