EPR and ^{19}F-ENDOR of 5,5-difluorocamphor Bound in Cytochrome P450cam Enzymatic Intermediates

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We report that cryoreduction of oxy and ferrous *cytrochrome P450cam* (P450cam) at 77 K generates EPR-active hydroperoxo-ferric and Fe(I) P450cam, respectively, which retain the conformations of their parent states.[1] Their EPR spectra reveal that the oxy-heme center exists in two major substates, Fig. 1. We have employed Q-band ^{19}F-ENDOR spectroscopy to examine 5,5-difluorocamphor (F2-camphor) bound to ferric P450cam, Fig. 2, as well as radiolytically cryoreduced to oxy and ferrous P450cam. Analysis of orientation-selective 2D ^{19}F-ENDOR spectra for both ferric and cryoreduced ferrous forms gives the ^{19}F-Fe distance as ca. 4.5 Å.

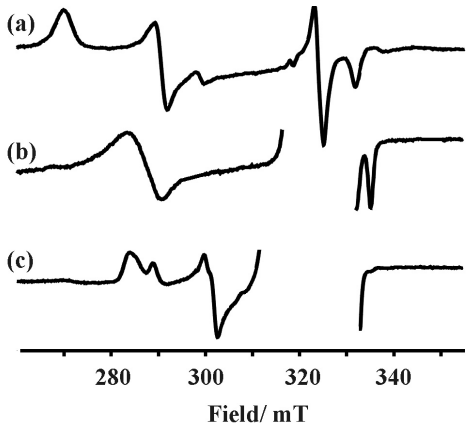

Fig. 1. 77 K X-band EPR spectra of (a) ferric P450cam, and cryoreduced (b) ferrous and (c) oxy P450cam in the presence of F2-camphor.

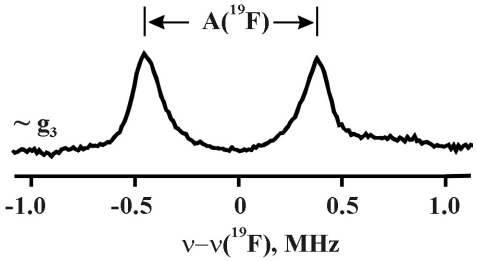

Fig. 2. ^{19}F-ENDOR of F2-camphor bound to ferric P450cam

1. R. Davydov et. al., *Biochemistry, 2005, 127, 1403-1413.*