EPR of Cu(II)-Methanobactin from Spent Media

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The EPR spectrum at the conventional microwave frequency (X-band), at a higher (Q-band), and a lower frequency (S-band) confirm the binding of cupric ion (see Fig.). Two observations distinguish the X-band spectrum of Cu-methanobactin, Cu-mb. First, the lines in the low field region (g_{ll}=2.23, A_{ll}=185 G) are broader than usual. This indicates more strain in the axial direction than observed from most type 2 cupric complexes. These lines in the g_{ll} region are sharper at a lower microwave frequency (S-band trace in Fig.). Second, in the X-band spectrum, there are lines at high field split by 16 G. These lines split by 16 G are evident in the S-band spectrum in the g_{l} region. The Q-band spectrum also has sharp lines on the high field side, which are attributed to the g_{l} region. Superimposed on the Cu-mb lines are five of six Mn lines and a free radical signal that are not detected at X- or S-band. Except for use as a field marker, these lines are not further considered. The 1st harmonic of the Q-band spectrum emphasizes the sharp lines. Since there are at least 10 lines split by 16 G that are resolved, and probably more unresolved lines in the spectra, these lines are attributed to superhyperfine lines due to nitrogen donor atoms in addition to protons that are close to the cupric ion. It is surmised that the cupric site is formed from three or four nitrogen donor atoms due to the number of lines resolved, and the g_{ll} value of 2.23 and the A_{ll} value of 185 G. A similar EPR spectrum is seen from a cupric bis-his site in a H129V mutant of nitrite reductase (Ellis et al., *Inorganic Chemistry* 43(24), 7591-93, 2004).