$[Cr(oxalate)_3]^{3-}$ as a Broadening Agent in Nitroxyl Spin Probe Studies

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Summary $[Cr(oxalate)_3]^{3-}$ broadens nitroxyl spin probe e.s.r. spectra several times more effectively than the broadening agents previously used.

NITROXYL spin probe studies often result in superimposed e.s.r. spectra representing the different environments probed by the nitroxyl radical. For example, spin probe investigations of the internal viscosity of cells result in the spectrum of the nitroxyl inside the cell superimposed on the spectrum (usually more intense) of the nitroxyl in the solution surrounding the cell. It has been observed that addition of a paramagnetic metal to the solution can broaden the e.s.r. spectrum of the nitroxyl in the outer solution, thus facilitating observation of the nitroxyl inside the cell.[1] Among the paramagnetic metal species which have been found useful for this purpose, the advantages of $[Fe(CN)_6]^{3-}$ have been emphasized.[1,2] We now report that $[Cr(oxalate)_3]^{3-}$ is more effective than $[Fe(CN)_6]^{3-}$ in broadening the nitroxyl e.s.r. spectrum.

The nitroxyl radical, 4-amino-2,2,6,6-tetramethylpiperidino-oxyl (commonly called tempamine) was used in these studies for comparison with prior results using

$[Fe(CN)_6]^{3-}$.[2] In 5 mM phosphate buffer adjusted to a final solution pH of 7·5, the e.s.r. signal of 10 mM tempamine is broadened to about the same extent by $[Cr(oxalate)_3]^{3-}$ (40 mM) as by $[Fe(CN)_6]^{3-}$ (80 mM). $[Cr(oxalate)_3]^{3-}$ (80 mM) broadens the nitroxyl e.s.r. spectrum such that it has only one third the amplitude of the spectrum of nitroxyl in the presence of $[Fe(CN)_6]^{3-}$ (80 mM). The pH, buffer, and concentrations of metal species and tempamine have been varied. For both $[Fe(CN)_6]^{3-}$ and $[Cr(ox)_3]^{3-}$ the effectiveness of the metal ion in broadening the tempamine e.s.r. spectrum is (a) greater at pH 7 than at pH 10, (b) greater in tris buffer than in phosphate buffer, (c) greater at low buffer concentrations than at high buffer concentrations, and (d) greater at low tempamine concentrations. However, for all the conditions examined $[Cr(ox)_3]^{3-}$ was two to three times more effective than $[Fe(CN)_6]^{3-}$ in broadening the tempamine e.s.r. spectrum.

$[Cr(ox)_3]^{3-}$ is very stable toward redox reactions which makes it more suitable for use with redox active substances than $[Fe(CN)_6]^{3-}$. Since lower concentrations of $[Cr(ox)_3]^{3-}$ than of $[Fe(CN)_6]^{3-}$ can effect the same broadening of the tempamine e.s.r. spectrum osmolality effects are diminished. Both features may be important in certain biological systems.

This work was supported in part by a grant from N.I.H. Helpful conversations with Professor S. P. Berg, University of Denver and Professor P. D. Morse, II, Wayne State University concerning application to cell studies contributed to this work.

(Received, 3rd July 1978; Com. 701.)

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