

Quantification of Dithiocarbamate-NO Complexes In Animal Tissues

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Iron-diethyl-dithiocarbamate complexes such as DETC or MGD are widely used for spin trapping of nitric oxide (NO) in biological systems like cell cultures or animal tissues. The three-line EPR spectrum with $a_N = 12.3$ G is characteristic of the NO-adduct.

Although, these spin traps are conventionally used in many laboratories, the quantification of NO in cells or tissues can be difficult. Dithiocarbamate has a strong affinity to copper, therefore the characteristic triplet of mononitrosyl-iron complex is often overlapped with the line of the copper-DETC. Therefore, special methods are needed for correct evaluation of NO-adduct spectra. In our study, we trapped nitric oxide with iron-DETC complex in tissues of control and streptozotocin-diabetic rats at an early stage of the disease. Tissue samples were measured in liquid nitrogen using an X-Band EPR spectrometer. In most cases, the NO-adduct spectrum was overlapped with a background line derived from the copper-DETC complex. To make the evaluation possible and more precise, we applied an EPR simulation program to eliminate the background line. A copper-DETC spectrum has been used as a template, and after a convergence test the program eliminated the background line from each spectrum by an active subtraction. After this procedure, the double integration of the NO triplets became possible and significant differences were found in liver and kidney 2 weeks, and in aorta 3 weeks after the onset of diabetes compared to control groups. This procedure allows a quantitative determination of NO-adduct spectra in organs of live animals even with abundant copper content.