Nitric Oxide Trapping Efficiencies of Water-Soluble Iron(III) Complexes with Dithiocarbamate Derivatives

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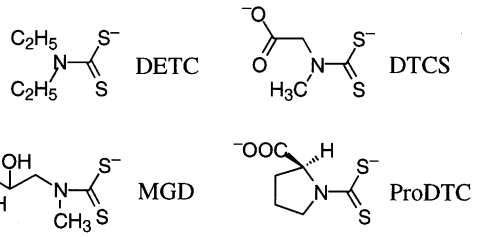
NO trapping efficiencies of three water-soluble iron(III) complexes with dithiocarbamate derivatives (DTC) are investigated by electron paramagnetic resonance spectroscopy. The obtained data show the dependence on the the structure of the substituent of -NRR' portion of DTC and on the solvent nature in which Fe-DTC complex are dissolved.

Nitric oxide (nitrogen monoxide, NO) is generated in biological cells and tissues and is an important mediator of various physiological and pathophysiological processes.[1,2] Owing to a small concentration (0.01-1μM) and a short half life (3-5 s for endothelial cells) of NO formed *in vivo*[3] the analyses of endogenous NO, which is generated from NO synthases, are very difficult.[4] Spin trapping technique combined with electron paramagnetic resonance (EPR) spectroscopy is one of the most powerful methods for directly measuring NO production in biological systems. Iron complexes with dithiocarbamate derivatives (DTC) are noted among the spin trapping reagents for NO because NO has a high affinity for the iron complexes and the resultant nitrosyl complexes exhibit intense triplet signal at room temperature, enabling *in vivo* determination of endogenous NO.[5,6]

Three iron complexes with DTC have been reported as the NO trapping reagents suitable for measuring NO production in biological systems. The Fe^{2+}-diethyldithiocarbamate (DETC) complex is the first one.[5] This complex, however, is insoluble in water; therefore, the Fe^{2+} complex of *N*-methyl-D-glucamine dithiocarbamate (MGD) has been proposed as a water soluble NO trap.[6,7] Expecting higher solubility in water than MGD, we used *N*-(dithiocarboxy)sarcosine (DTCS) as a ligand. Since DTCS contains an anionic carboxyl group even if it ligates to iron ion, Fe-DTCS complex is indeed more soluble in water than Fe-MGD complex. Owing to its high solubility the NO-Fe(DTCS)$_2$ complex can be used as a spin probe for EPR imaging of small animals.[8,9] The Fe-DTCS complex can be used certainly as an NO trapping reagent, and so we succeeded in detecting the EPR spectrum and obtaining the EPR image of the nitrosyl complex derived from the endogenous NO and the Fe-DTCS complex in the abdominal region of lipopolysaccharide-treated mice.[10]



We have been studying further the analytical method of NO using Fe-DTC complexes. Although these complexes are capable of trapping the endogenous NO, the method of the quantification of produced NO has not been established. Not only calculation of the concentration of the nitrosyl-Fe complex observed in the EPR spectrum but evaluation of the NO trapping efficiency (% yield) of the Fe-DTC complex is important for quantification of NO produced in a sample. If the trapping efficiency is known, we can estimate the amount of the NO production. This is useful for *in vitro* measurements, specially for cultured cells such as macrophages. In the course of our study, moreover, we find Fe^{3+}-DTC complexes also react with NO to form NO-Fe^{2+}(DTC)$_2$ complexes. By using the Fe^{3+} complexes, we can prepare the traps more easily under aerobic condition. We investigated, therefore, the NO trapping efficiency of three water soluble Fe^{3+}-DTC complexes in four medium.

The used DTC were DTCS, MGD and newly synthesized L-prolinedithiocarbamate (ProDTC). MGD sodium salt was synthesized according to the mothod of Shinobu *et al.*[11] DTCS disodium salt and ProDTC diammonium salt were synthesized by a modified method of the literature.[11] The procedure for EPR measurements was as follows: DTC with $FeCl_3$ was aerobically dissolved in medium ([DTC]/[Fe]=3). An aliquot (10 μL) of the NOC-5[12] (Dojindo) solution (50 mM in 0.1 N NaOHaq.) was then added to 4990 μL of the Fe(DTC)$_3$ solution (final Fe and NOC-5 concentrations, [Fe] = 1 mM, [NOC-5] = 0.1 mM). Half a minute after addition of the NOC-5 solution, the sample solution was taken in a capillary tube (75 mm in length, 46 μL inner volume) and then inserted into a normal quartz EPR cell (o.d. = 5 mm). The EPR spectrum was recorded at five minutes intervals from 5 to 120 min after addition of the NOC-5 solution at room temperature. The final pH of the solution increased to 0.1 - 0.3 units from the initial value.

The NOC-5 gradually releases NO when it is added to neutral buffer or solution. Figure 1 shows the time-dependent spectral change of EPR of Krebs bicarbonate buffer (pH 7.40) solution containing Fe(DTCS)$_3$ and NOC-5. A distinct triplet EPR spectrum (a^N = 1.27 mT and g_{iso} = 2.040), which is identified as

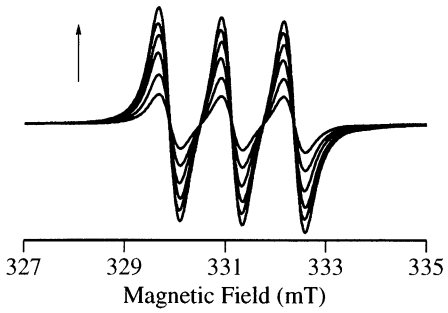


Figure 1. Time-dependent changes in the X-band EPR spectra of the [NO-Fe(DTCS)$_2$]$^{2-}$ in Krebs bicarbonate buffer solution at room temperature. For simplicity, the spectra at 20, 40, 60, 80, 100, and 120 min after addition of the NOC-5 solution are shown. Instrument settings: microwave power, 60 mW and modulaton width, 0.32 mT.

Table 1. Comparison of the NO trapping efficiency and the chemical properties of iron complex with DTC

dithiocarbamates	parent amine	$pK_a(BH^+)$	NO Trapping Efficiency (% Yield)[a]				IR (KBr, cm^{-1})		EPR[b]	
			Krebs[c]	PBS[d]	HBSS[e]	Tris/HCl[d]	ν_{N-O}	ν_{C-N}	g_{iso}	a^N (mT)
PDTC[f]	Pyrrolidine	11.11[g]					1696	1503	2.041	1.260
DETC	*N,N*-Diethylamine	10.93[h]					1687	1505	2.039	1.285
ProDTC	L-Proline	10.38[i]	30	5	49	82	1714[j]	1497	2.040	1.265
DTCS	*N*-Methylglycine	9.97[i]	60	40	48	95	1693	1529	2.038	1.278
MGD	*N*-Methyl-D-glucamine	9.56[k]	55	35	56	95	1708	1519	2.039	1.274

[a]Calculated from the EPR signal at two hours after addition of NOC-5 solution. Data are means from $n = 3$. S.E.was < 10 % in all cases. [b]Data obtained from the spectra of DMSO solution. Both water soluble and water insoluble complexes are dissolved in DMSO. [c]pH 7.40. [d]0.1 M, pH 7.40. [e]pH 7.20. [f]Pyrrolidinedithiocarbamate. [g]Ref. 15. [h]Ref. 16. [i]Ref. 17. [j]A peak at 1834 cm^{-1} is also observed. [k]This work.

the $[NO\text{-}Fe^{2+}(DTCS)_2]^{2-}$ complex,[8] is observed and its intensity gradually increases. The appearance of the EPR signal indicates that the nitrosyl iron complex is formed by the reductive nitrosylation.[14] The scrutiny of the mechanism of the reductive nitrosylation is now under way. Other two DTC complexes show similar behavior, except in signal intensity (Figure 2). This difference suggests that the NO trapping efficiencies of the Fe-DTC complexes depend on the used ligand. Interestingly the NO trapping efficiencies also depend on the used medium. The results are summarized in Table 1 with the IR and EPR parameters.

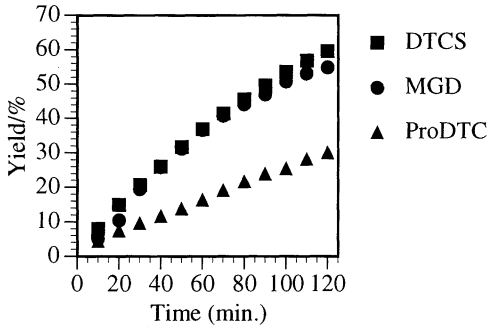


Figure 2. Time courses of the trapping efficiency (% yield) of NO released from NOC-5. For calculation of the NO trapping efficiency the amplitude of the first low-field signal of NO-Fe-DTC complex was calibrated by comparison with the amplitude of the EPR signal of the standard solution of $NO\text{-}Fe(DTCS\cdot Na)_2$ powder synthesized by a modified method of the literature.[13]

The differences in the trapping efficiency and the spectroscopic parameters are ascribed to the -NRR' portion of the dithiocarbamate ligand. With regard to the DTC having dialkyl substituents, it was reported that the major property of the -NRR' portion of the DTC is its "strong mesomeric electron-releasing effect" which may be quantified by the pK_a value of the protonated form of the parent secondary amine, $H_2NRR'^+$.[18,19] The increase in pK_a results in the increase in the double bond character of C-N bond, and results in weakening of the N-O bond strength. As to the three DTC with a polar group in R, these correlation cannot be seen. Further systematic studies are needed to relate the NO trapping efficiency with the electronic property of the ligand.

In summary, the data on the NO trapping efficiency provide us with a novel analytical method which can evaluate the NO production. The quantification of the NO production makes the analytical method using the Fe-DTC complexes more useful. Further the use of the Fe^{3+} salts as an iron source makes the preparation of the traps easy. We believe that this method is applicable not only to the *in vitro* system but to the *in vivo* system.

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