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Ab initio *g*-tensor calculations of the thioether substituted tyrosyl radical in galactose oxidase

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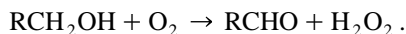
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Abstract

The tyrosyl radical in galactose oxidase is covalently cross-linked to a neighboring cysteine residue through a thioether bond. The role of this sulfur cross-link has been discussed ever since the crystal structure of the enzyme was solved. In the present work, the ab initio multiconfigurational linear response method is applied to calculate the *g*-tensor of unsubstituted and thioether substituted phenoxyl radicals. In contrast to some previous interpretations, but in agreement with recent EPR measurements, we find that the sulfur substitution induces only minor shifts in the *g*-tensor components. The spin distribution retains the odd-alternant pattern of the unsubstituted radical and only a small amount of spin is localized to the sulfur center. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Galactose oxidase (GO) catalyzes the two-electron oxidation of a large number of primary alcohols to their corresponding aldehydes, coupled with the reduction of dioxygen to hydrogen peroxide [1,2]:



GO contains two one-electron redox centers, a copper center and a ligating tyrosyl radical. The crystal structure of the active form of GO has been determined at pH values 4.5 and 7.0 [3]. As seen in Fig. 1, the coordination of copper is essentially square-pyramidal with Tyr495 in axial position, and

Tyr272, His581, His496 and a water or acetate to be replaced by a substrate in equatorial positions. Tyr272 exhibits the unusual feature in being covalently cross-linked, at the *ortho*-position to the phenol OH, to a neighboring cysteine residue (Cys228) through a thioether bond [3]. The protein radical interacts antiferromagnetically with the copper ion, resulting in an EPR silent species. An EPR spectrum is, however, detected in the apo-enzyme, where the copper atom has been removed.

The effects of the thioether bond on the properties of the tyrosine have been subject to discussion. It has been speculated that this thioether bond is in part responsible for the 0.5–0.6 V lowering of the oxidation potential of this species compared to normal tyrosyl [1,4]. Based on a series of EPR and ENDOR experiments on both the apo-enzyme and the model alkylthio substituted phenoxyl radicals, it was shown

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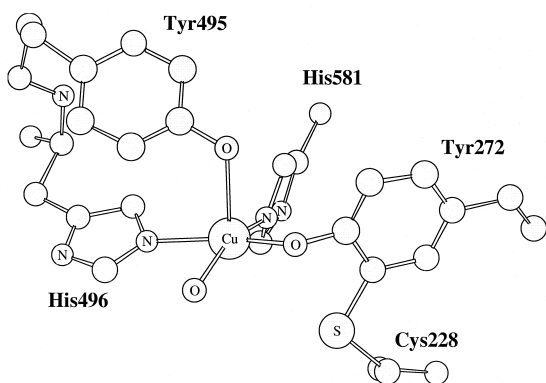


Fig. 1. Crystal structure of the active site of galactose oxidase.

by Babcock and co-workers [4] that the sulfur cross-link does not severely perturb the spin distribution of the tyrosyl radical. Comparing the spectra of methylthio substituted radicals with unsubstituted ones, Babcock et al. detected no major shift in the g -tensors, which otherwise would be expected for sulfur based radicals. The interpretation of this missing shift was that the sulfur substituent only has a small amount of spin, not sufficient to significantly perturb the spin density distribution of the tyrosyl radical. Gerfen et al. combined high-field EPR experiments with density functional calculations (BLYP with numerical basis set) to study this radical in apo-galactose oxidase and model radicals generated by UV-photolysis [5]. Spectra from the sulfur-substituted tyrosyl radicals showed very close similarities, exhibiting nearly axial symmetry of the g -values, in contrast to spectra from unsubstituted tyrosyls, in e.g. ribonucleotide reductase (RNR) and single crystals, which instead exhibit essentially rhombic g -values. The simulated g -tensor components from the experiment on apo-galactose oxidase by Gerfen et al. are $g_x = 2.0074$, $g_y = 2.0064$, and $g_z = 2.0021$ [5]. These values are almost identical to the ones obtained by Babcock et al. ($g_x = 2.0076$, $g_y = 2.0066$, and $g_z = 2.0023$) [4], although Babcock et al. in the simulations used a g_x principal axis parallel to the CO bond, while Gerfen et al. assumed a 23° rotation from that bond.

The calculations by Gerfen et al. were only capable to reproduce trends in the g -tensors. For instance, the difference in the g_x value between RNR tyrosyl (unsubstituted) and apo-GO tyrosyl (thioether

substituted) is experimentally determined to 0.002 (2.0091 vs. 2.0074), whereas the calculated difference between cresyl and *o*-methylthio-cresyl was 0.011 (2.0170 vs. 2.0060). The BLYP calculations also indicated that the thioether substitution caused substantial delocalization of the spin density onto the sulfur center (calculated to 0.28).

Using several more accurate DFT functionals (B3LYP, PWP86, and B3P86), it was recently shown that hyperfine couplings and spin distributions are very little affected by the presence of the thioether bond [6,7]. The sulfur center was calculated to possess around 0.12 of the unpaired spin, maintaining the odd-alternant spin pattern of the unsubstituted radical. Hence, the picture of the sulfur-substituted tyrosyl radical not being much different from normal tyrosyl was confirmed.

In the present work, we have applied ab initio multiconfigurational linear response methodology to calculate the g -tensors of the sulfur substituted and unsubstituted tyrosyl radicals in order to shed further light on the conflicting interpretations. The main conclusion is that the sulfur substitution only induces small changes in the g -tensor, adding yet another piece of support to the original picture by Babcock et al. Effects of sulfur substitutions in *meta* and *para* positions were also calculated.

2. Computational details

The cysteine-linked tyrosyl radical in galactose oxidase was modeled by *o*-thiophenoxyl (Fig. 2).

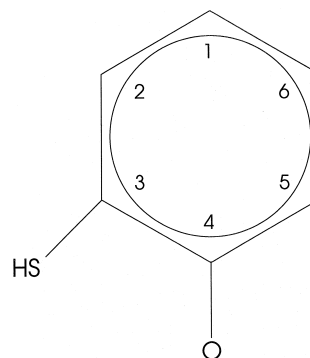


Fig. 2. Numbering scheme of the phenoxyl radical with sulfur substitution in *ortho* position (phenoxyl-S).

Phenoxyl has previously proven fully adequate to model the electronic structure of the tyrosyl radical [8]. In particular, hyperfine coupling constants have been successfully reproduced [6,9].

EPR g -tensor components were calculated with the linear response method implemented in a local version of the DALTON quantum chemistry program [10,11]. This method gives g -values consistent with experiments for diatomic triplets and substituted benzene radicals [12,13]. It has been shown that the multiconfigurational approach is essential for phenoxyl radical g -values [13], and therefore CASSCF wavefunctions were used in this work.

The g -tensor components are calculated as a shift Δg from the free electron value, $g_e = 2.002319$. The g -shift is dominated by the spin–orbit coupling (SOC) and orbital–Zeeman (OZ) cross terms ($\Delta g_{\text{SOC/OZ}}(1e), \Delta g_{\text{SOC/OZ}}(2e)$).

$$\Delta g_{\text{SOC+OZ}}^{\text{ab}}(1e) = \frac{\alpha^2}{S} \sum_n \frac{\langle \Psi_0 | \sum_i \sum_K \frac{Z_K}{r_{iK}^3} l_{iK}^a s_{z,i} | \Psi_n \rangle \langle \Psi_n | \sum_i l_{iC}^b | \Psi_0 \rangle}{E_0 - E_n}, \quad (1)$$

$$\Delta g_{\text{SOC+OZ}}^{\text{ab}}(2e) = -\frac{\alpha^2}{S} \sum_n \frac{\langle \Psi_0 | \sum_i \sum_{j \neq i} \left(\frac{l_{ij}^a}{r_{ij}^3} + 2 \frac{l_{ji}^a}{r_{ij}^3} \right) s_{z,i} | \Psi_n \rangle \langle \Psi_n | \sum_i l_{iC}^b | \Psi_0 \rangle}{E_0 - E_n}, \quad (2)$$

where the sum is over excited states Ψ_n of same spin as the ground state Ψ_0 . E denotes the energy and l_{iK}, l_{iC} , and l_{ij} denote angular momentum elements. Relativistic mass (Δg_{RMC}) and gauge corrections ($\Delta g_{\text{GC}}(1e), \Delta g_{\text{GC}}(2e)$) provide minor contributions. Calculated g -shifts in this work were based on the following corrections [14]

$$\Delta g^{\text{ab}} = \Delta g_{\text{RMC}} \delta^{\text{ab}} + \Delta g_{\text{GC}}(1e) + \Delta g_{\text{SOC/OZ}}(1e) + \Delta g_{\text{SOC/OZ}}(2e). \quad (3)$$

The two-electron gauge correction was not included. This contribution is not expected to influence the g -values significantly, except in those cases when the g -shift is close to zero. Excited state properties, such as energies, spin–orbit coupling and angular momentum matrix elements, are solved from the response equations. Excited states are thereby calculated only implicitly. In this way, the error in having to truncate

the summation over excited states is avoided, which is the major advantage with the response function technology. Earlier works by the present authors describe the different contributions to g -shifts in more detail [12,13].

The geometries of the phenoxyl radical and its sulfur substitutions were calculated with the B3LYP/6-31G** method as implemented in the GAUSSIAN98 program [15]. The CASSCF/cc-pVDZ spin densities were calculated with GAUSSIAN98, revision A.7. The g -tensors were calculated with cc-pVDZ basis set using the MCSCF linear response method in the DALTON program [11]. Choices of the ground state symmetries and active spaces (CAS) were made on the basis of the UNO-CAS method which is founded on UHF natural orbital population analysis [16]. The radicals were allocated a Π ground state symmetry with 7 electrons in 7 active orbitals. These active space and basis set parameterizations follow in part earlier experience with studies on sulfur centered radicals [17].

3. Results

The spin distribution of the unsubstituted phenoxyl radical exhibited the well-characterized odd-alternant pattern (Table 1), in accordance with previous theoretical work [6,7,9]. As seen in Table 1, the CASSCF spin density had values approximately intermediate to the DFT results. The spin distribution of the sulfur-substituted radical (phenoxyl-S) was found to be essentially equivalent to the unsubstituted radical. Only a small part of the spin (0.03) was localized to the sulfur atom (Table 1). Hence, the CASSCF method assigned less spin density on the sulfur atom compared with the B3LYP and PWP86 methods (0.11 and 0.15, respectively). The atomic orbital contribution to the singly occupied molecular orbital (SOMO) obtained from the MCSCF calculation followed the same pattern. That is, the unpaired electron was located mainly in oxygen and alternating carbon orbitals and the sulfur atom provided only a minor contribution to SOMO. These results are contradictory to the results of Gerfen et al. There, the sulfur atom was found to provide the largest atomic orbital contribution to SOMO and a

Table 1

DFT and CASSCF spin densities of the sulfur substituted (phenoxy-S) and the unsubstituted phenoxy radical (the numbering scheme is given in Fig. 2)

Atom	Phenoxy-S			Phenoxy		
	B3LYP [6]	PWP86 [6]	CASSCF	B3LYP [6]	PWP86 [6]	CASSCF
C1	0.34	0.28	0.38	0.40	0.36	0.38
C2	−0.16	−0.09	−0.14	−0.16	−0.10	−0.13
C3	0.28	0.21	0.33	0.31	0.26	0.30
C4	−0.06	0.02	−0.10	−0.11	−0.03	−0.11
C5	0.23	0.18	0.26	0.31	0.25	0.30
C6	−0.11	−0.03	−0.12	−0.16	−0.09	−0.13
O	0.35	0.28	0.35	0.43	0.37	0.39
S	0.11	0.15	0.03	—	—	—

large amount of the spin (0.28) was located to the sulfur center [5].

Calculated g -values for the phenoxy-S and phenoxy radicals are presented together with experimental data in Table 2. The g -tensor components of the phenoxy radical were $g_x = 2.0087$, $g_y = 2.0050$, and $g_z = 2.0025$. That is, the g -tensor was essentially rhombic with g_x directed along the CO bond, g_y in the molecular plane, and g_z out of the plane. As reported in earlier work [18], the g_x component is strongly dependent on the CO bond length; a longer CO bond length gives a higher g_x value. The g_x component is also influenced by hydrogen bonding to the phenoxy oxygen. Geometric and environmental factors may thus explain the experimental variations in the g_x component of phenoxy in different molecular systems [4,5,19–21]. Despite differences in g_x , the g -tensor of the phenoxy radical has

a rhombic structure which was also obtained in the calculations.

The principal axes of the phenoxy-S g -tensor were found to be rotated by 8° in the molecular plane (Fig. 3). The x -axis was shifted away from the sulfur atom and was no longer collinear with the C–O bond. Gerfen et al. reported the same trend in the rotation of the g -tensor principal axes. However, they found a larger deviation from the unsubstituted radical (23°) [5], consistent with the larger spin density they found at the sulfur center.

For phenoxy-S, the calculated g -values were $g_x = 2.0079$, $g_y = 2.0052$, and $g_z = 2.0025$. The largest shift induced by the sulfur substitution is seen for the g_x component (2.0087 vs. 2.0079). The main contribution to g_x originates from the oxygen $n\pi^*$ excitation. Sulfur substitution of the phenoxy radical produced a blue shift of this excitation. Excitation

Table 2

Experimental (expt.) and calculated (calc.) g -tensors of unsubstituted and sulfur-substituted tyrosyl radicals in proteins and model systems

System		g_x	g_y	g_z	g_{av}	Ref.
apo-galactose oxidase	expt.	2.0076	2.0066	2.0023	2.0055	[4]
apo-galactose oxidase	expt.	2.0074	2.0064	2.0021	2.0053	[5]
Methylthio-cresyl	expt.	2.0072	2.0062	2.0019	2.0051	[5]
Methylthio-cresyl	calc.	2.0060	2.0044	2.0023	2.0042	[5]
Phenoxy-S	calc.	2.0079	2.0052	2.0025	2.0052	this work
<i>E. coli</i> ribonucleotide reductase	expt.	2.0091	2.0046	2.0022	2.0053	[5]
<i>E. coli</i> ribonucleotide reductase	expt.	2.0087	2.0042	2.0020	2.0050	[8]
Phenoxy	expt.	2.0072	2.0043	2.0024	2.0046	[21]
Cresyl	calc.	2.0170	2.0033	2.0023	2.0075	[5]
Phenoxy	calc.	2.0087	2.0050	2.0025	2.0054	this work

energies were 2.17 eV for the unsubstituted radical and 2.49 eV for the *ortho* substitution. Spin-orbit coupling (SOC) and angular momentum (AM) matrix elements of the $n\pi^*$ excitation in the *ortho* radical were strikingly lower (SOC = 19.16 cm^{-1} and AM = 0.43) compared with the phenoxyl radical (SOC = 35.38 cm^{-1} and AM = 0.75). Hence, sulfur substitution in *ortho* position induced a reduced contribution from the $n\pi^*$ excitation to g_x . The lower g_x value of the phenoxyl-S radical may partially be explained by the higher excitation energy together with the lower spin-orbit coupling and angular momentum matrix elements of the $n\pi^*$ excitation. However, g_x of this radical obtained an appreciable contribution from the second excited state, which was not observed for the unsubstituted radical.

The experimental difference in g_y is 2×10^{-3} , in average (Table 2). The larger g_y values are observed for the thioether substituted radicals. The calculated g_y component, however, remained basically unaltered upon sulfur substitution in *ortho* position. To further elucidate the effect of sulfur substitution of the phenoxyl radical, the EPR g -values of thiol-substituents in *meta* and *para* positions were also calcu-

Table 3

Calculated g -values for the phenoxyl and sulfur substituted phenoxyl radicals with substitutions in *ortho*, *meta* and *para* positions

	g_x	g_y	g_z	g_{av}
Phenoxyl	2.0087	2.0050	2.0025	2.0054
<i>Ortho</i>	2.0079	2.0052	2.0025	2.0052
<i>Meta</i>	2.0081	2.0047	2.0025	2.0051
<i>Para</i>	2.0078	2.0059	2.0025	2.0054

lated. The g -tensors of these two substitutions were $g_x = 2.0081/2.0078$, $g_y = 2.0047/2.0059$, and $g_z = 2.0025/2.0025$ (Table 3). The calculated g_y values varied significantly, depending on the position of the sulfur substituent, whereas the g_x component showed less variations.

4. Discussion

Calculations in this work showed that the g -tensor of the sulfur substituted phenoxyl radical followed the same pattern as other phenoxyl type radicals [22,23]. This pattern is significantly different from sulfur centered radicals. Radicals with the spin density concentrated on sulfur atoms induce a pronounced shift of the g -tensor components [24]. The cysteine radical, for example, has a characteristic g -tensor, $g_x = 2.251$, $g_y = 2.004$, $g_z = 1.985$, with the major component g_x directed along the C–S bond [25]. Another example is the methyl disulfide radical $\text{CH}_3\text{SS}^\cdot$, for which calculations with the present method yielded $g_x = 2.0628$, $g_y = 2.0281$, $g_z = 2.0023$ [17]. These values are typical for perthiyl RSS^\cdot radicals [24].

The spin density distributions were essentially undisturbed upon sulfur substitution. That is, the alternant spin density pattern was maintained and only a small part of the spin density was transferred to the sulfur atom. This pattern was clearly reflected in the calculated g -tensor components. The in-plane components showed small variations while the out of plane component g_z remained basically unaltered. The difference in the g_x component between the phenoxyl and phenoxyl-S radicals was 0.001 (2.0087 vs. 2.0079). The lower g_x of phenoxyl-S was traced to substantially lower spin-orbit coupling and angu-

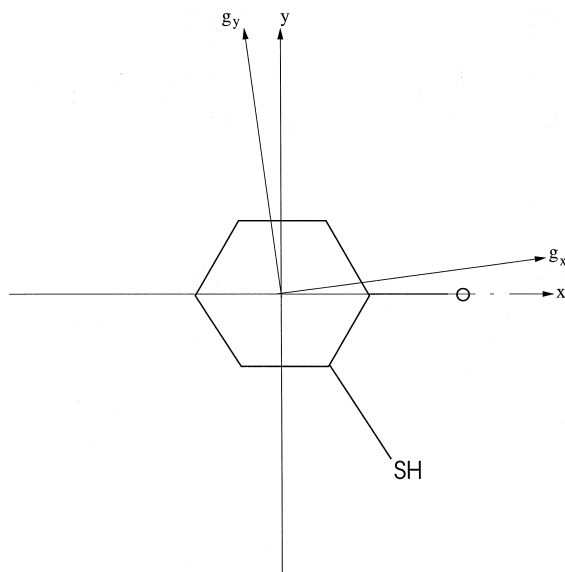


Fig. 3. The principal axes of the g -tensor in phenoxyl-S compared with the molecular coordinate system. The g_x component of the phenoxyl radical was aligned with the CO bond whereas g_x of phenoxyl-S was rotated 8° away from the CO bond.

lar momentum matrix elements of the $n\pi^*$ excitation. The higher energy of this excitation also contributed to the decreased g_x value. It has, however, been shown in earlier g -value calculations that contributions from higher excited states are important to obtain accurate magnitudes, and even the correct sign, of the g -shift [12]. Contribution to g_x from only the lowest excited states would produce a more pronounced decrease of the g -value than what was observed. This may explain deviations between experimental results and g -values calculated with methods which comprise truncations of the sum-over-states values, something which thus is avoided by the present response theory method.

In conclusion, the sulfur atom of the thioether substituted tyrosyl radical induces a small shift of the g -tensor components towards less rhombic values. However, the g -tensor maintained basically the same pattern as for other phenoxy type radicals. For the case of galactose oxidase, this is yet another indication that the cysteine cross-link has no major electronic influence on the tyrosyl radical.

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References

- [1] J. Stubbe, W.A. van der Donk, *Chem. Rev.* 98 (1998) 705.
- [2] J.P. Klinman, *Chem. Rev.* 96 (1996) 2541.

- [3] N. Ito, S.E.V. Phillips, C. Stevens, Z.B. Ogel, M.J. McPherson, J.N. Keen, K.D.S. Yadav, P.F. Knowles, *Nature* 350 (1991) 87.
- [4] G.T. Babcock, M.K. El-Deeb, P.O. Sandusky, M.M. Whittaker, J.W. Whittaker, *J. Am. Chem. Soc.* 114 (1992) 3727.
- [5] G.J. Gerfen, B.F. Bellew, R.G. Griffin, D.J. Singel, Ch.A. Ekberg, J.W. Whittaker, *J. Phys. Chem.* 100 (1996) 16739.
- [6] F. Himo, G.T. Babcock, L.A. Eriksson, *Chem. Phys. Lett.* 313 (1999) 374.
- [7] K.E. Wise, J.B. Pate, R.A. Wheeler, *J. Phys. Chem. B* 103 (1999) 4764.
- [8] S. Un, M. Atta, M. Fontecave, A.W. Rutherford, *J. Am. Chem. Soc.* 117 (1995) 10713.
- [9] F. Himo, A. Gräslund, L.A. Eriksson, *Biophys. J.* 72 (1997) 1556.
- [10] O. Vahtras, B. Minaev, H. Ågren, *Chem. Phys. Letters* 281 (1997) 186.
- [11] T. Helgaker et al., DALTON release 1.0 (1997), an electronic structure program.
- [12] M. Engström, B. Minaev, O. Vahtras, H. Ågren, *Chem. Phys.* 237 (1998) 149.
- [13] M. Engström, O. Vahtras, H. Ågren, *Chem. Phys.* 243 (1999) 263.
- [14] J.E. Harriman, *Theoretical Foundations of Electron Spin Resonance*, Academic Press, New York, 1978.
- [15] M.J. Frisch et al. GAUSSIAN 98, Revision A.3, Gaussian, Inc., Pittsburgh, PA, 1998.
- [16] J.M. Bofill, P. Pulay, *J. Chem. Phys.* 90 (1989) 3637.
- [17] M. Engström, H. Ågren (submitted).
- [18] M. Engström, F. Himo, A. Gräslund, O. Vahtras, B. Minaev, H. Ågren, submitted (unpublished).
- [19] P.J. van Dam, J.-P. Willems, P.P. Schmidt, S. Pötsch, A.-L. Barra, W.R. Hagen, B.M. Hoffman, K.K. Andersson, A. Gräslund, *J. Am. Chem. Soc.* 120 (1998) 5080.
- [20] E.L. Fasanella, W. Gordy, *Proc. Natl. Acad. Sci. USA* 62 (1969) 299.
- [21] A.Yu. Bresgunov, A.A. Dubinsky, O.G. Poluektov, Ya.S. Lebedev, *Mol. Phys.* 75 (1992) 1123.
- [22] R.V. Lloyd, D.E. Wood, *J. Am. Chem. Soc.* 96 (1974) 659.
- [23] P. Neta, R.W. Fessenden, *J. Phys. Chem.* 78 (1974) 523.
- [24] D.J. Nelson, R.L. Petersen, M.C.R. Symons, *J. Chem. Soc. Perkin Trans. II* (1977) 2005.
- [25] H.C. Box, H.G. Freund, E.E. Budzinski, *J. Chem. Phys.* 45 (1966) 809.