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CAN GEOMETRICAL DISTORTIONS MAKE A LACCASE CHANGE COLOR FROM BLUE TO YELLOW?

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ABSTRACT. Laccases contain a blue mononuclear copper center known as 'type-1', and thought to be the primary electron acceptor from organic substrates during the catalytic cycle. A small group of laccases are also known that lack the 600 nm band and hence the blue color ("yellow laccases"). We report on the use of semiempirical (ZINDO/S-CI) calculations in order to simulate UV-vis spectral parameters for the laccase type 1 copper, attempting to assign geometrical and electronic structure elements that may control the color of this site. The ~600-nm band of the type 1 copper is confirmed to arise mainly from sulfur-to-copper charge transfer, and strong distortions allowing for its displacement by more than 200 nm and/or its dissolution are identified.

Keywords: *laccase, type 1, copper, UV-vis, EPR, DFT, ZINDO/S*

INTRODUCTION

Laccase enzymes contain a mononuclear copper centre known as 'type-1' (Figure 1) [1]. This site is thought to be the primary electron acceptor from organic substrates. This site is characterized by a 610 nm band in UV-vis spectra and a small hyperfine coupling constant in electron paramagnetic resonance (EPR) spectra [2]. A small group of laccases are also known that lack the 600 nm band and hence the blue colour ("yellow laccases") [3-5]. Several authors postulated that the yellow laccases are formed by modification of the blue form with low-molecular-weight lignin decomposition products [5, 6], even though there's no experimental proof yet. Numerous yellow laccases have been purified from a number of organisms such as the common mushroom *Agaricus biosporus* [7], phytopathogenic ascomycete *Gaeumannomyces graminis* [8], *Schizophyllum commune* [9], *Phlebia radiata* [5], *Phellinus ribis* [10], and *Pleurotus ostreatus* D1 [3], *Ph. tremellosa* [5].

We have recently characterized a "yellow" laccase, whose type 1 copper centre lacks the characteristic 610 nm band, but does still show an EPR spectrum typical of a blue laccase is shown in detail [11]. We now report

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on the use of density functional theory (DFT) and semiempirical (ZINDO/S-CI) calculations in order to simulate spectral parameters for the laccase type 1 copper as found in blue and in yellow laccases, attempting to assign geometrical and electronic structure elements that may control the trends observed experimentally in EPR coupling constants and in UV-vis spectra.

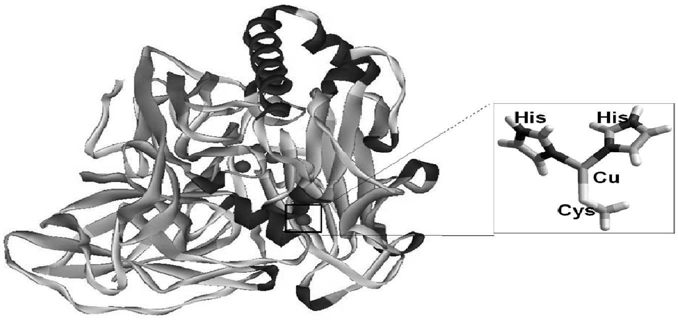
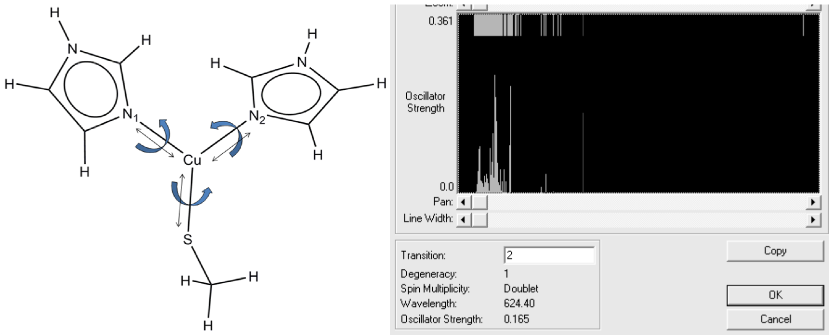


Figure 1. General structure of laccases (based on pdb ID 2Q9O), highlighting the four copper ions and the coordination environment around the type 1 copper center.

RESULTS AND DISCUSSION

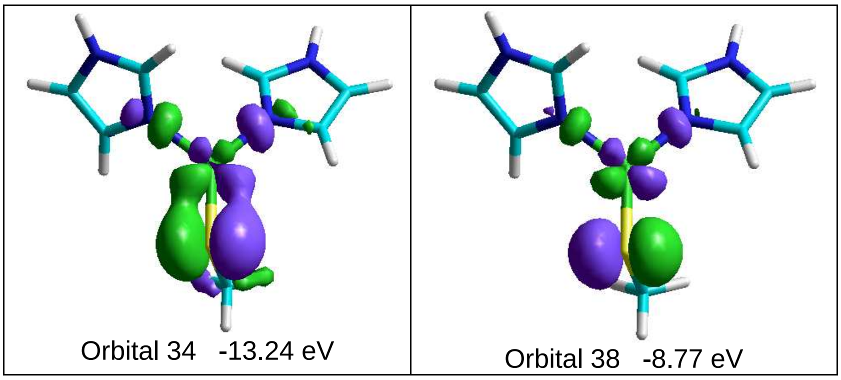
Figure 2 shows the model employed in the present work for the type 1 copper. ZINDO/S- configuration interaction calculation calculations, with a 20 eV excitation window allowed prediction of a single band in the visible region of the UV-vis spectrum of this species, as also shown in Figure 2. This band had a wavelength of 624 nm and an oscillator strength of 0.16, both of which are in reasonable agreement with experimental data known for laccases (a band at ~600 nm with sulphur-to-copper charge-transfer character).



Transition: 2
Degeneracy: 1
Spin Multiplicity: Doublet
Wavelength: 624.40
Oscillator Strength: 0.165

Figure 2. Model of the type 1 copper of laccases, employed in the present work. Arrows indicate bonds around/along which distortions were performed. Right panel: ZINDO/S-CI spectrum computed for this model prior to applying distortions

The 624 nm band shown in Figure 2 was found to arise dominantly from a single electronic transition. Namely, involved were the CI states 1 (with a contribution of 0.99689 from the ground state) and 3, with a contribution of -0.82884 from an excitation for which the two orbitals responsible for it are shown in Figure 3. These are the π and π^* orbitals of the Cu-S bond.


Orbital 34 -13.24 eV

Orbital 38 -8.77 eV

Figure 3. The main orbitals responsible for the blue color of the laccase type 1 center, according to ZINDO/S-CI calculations.

The oscillator strength relates to the experimentally-measured extinction coefficient; values shown here between 0.01 and 0.1 equate to extinction coefficients in the range of thousands. Analyzing the effect of distortions on the 624-nm band of the type 1 copper site, we note that even 0.1-Å changes in the Cu-S bond length are predicted to lead to clearly detectable shifts in the maximum (up to 100 nm). In fact, elongation to 2.48 Å (a value not uncommon for other metal-sulphur bonds in proteins) would take the sulphur-to-copper charge transfer up by ~200 nm in energy, to a region already dominated by histidine-to-copper charge transfer bands from the other two ligands (not to mention overlapping absorbance of the same from the other three copper ions in the protein, all three of which have their own histidine ligands). Another distortion with a strong effect is rotation around the Cu-S bond; this was expected, since such rotation will affect the efficiency of the π interaction between the copper and the sulphur – an interaction which as shown above is essential for the 624-nm band computed in the initial structure. Thus, rotation to 51º (60º relative to the initial value) would take the charge-transfer band down in energy by ~ 200 nm, as would rotation to 231º - in both cases with a decrease in extinction coefficient. Rotation to 81º (only 30º relative to the initial structure) and to 351º are predicted to still allow for bands in the 600-nm region, only with extinction coefficients ~20 times lower than those of the initial structure.

Table 1. Computed absorption maxima (nm) for the type 1 laccase centre, as seen in a crystal structure of a “blue” site (“initial”, pdb 1Q9O) and then subjected to compressions, elongations or rotations around bonds indicated in Figure 2. Numbers in the first column indicate the values taken by the parameters modified (bond lengths in Å, torsion angles in º). The initial values of these parameters were 2.18 Å Cu-S, 2.01 Å Cu-N1,1.94 Å Cu-N2, 111º CSCuN, 129º CN1CuS and 155º CN2CuS. Also shown are the energies (eV) of the two orbitals mainly responsible for the band computed to occur at 624 nm in the “initial “structure.; “n.a.”: a single orbital displaying the given character was no longer identifiable.

	Absorption maxima	Oscillator strength	π	π*
intial	624	0.165	-13.243	-8.769
Cu-S 1.98	448	0.073	-13.787	-8.252
Cu-S 2.08	514	0.133	-13.528	-8.537
Cu-S 2.28	775	0.161	-12.977	-8.952
Cu-S 2.38	840	0.116	-12.788	-9.080
Cu-S 2.48	432	0.021	n.a.	-8.946
Cu-S 2.58	768	0.045	n.a.	-9.254
Cu-S 2.68	790	0.021	n.a.	-9.400
Cu-N11.91	621	0.163	-13.103	-8.618
Cu-N1 2.11	611	0.168	-13.368	-8.896
Cu-N1 2.21	617	0.152	-13.459	-9.018
Cu-N1 2.31	597	0.151	-13.552	-9.120
Cu-N1 2.41	594	0.142	-13.625	-9.227
Cu-N2 2.04	617	0.157	-13.371	-8.894
Cu-N2 2.14	591	0.156	-13.489	-8.998
Cu-N2 2.24	599	0.137	-13.566	-9.111
Cu-N2 2.34	572	0.135	-13.666	-9.200
Cu-N2 2.44	578	0.122	-13.724	-9.301
CSCuN 51	806	0.095	-13.017	-8.893
CSCuN 81	505, 507, 583	0.001, 0.005, 0.003	-12.802	-9.013
CSCuN 171	610	0.002	-12.598	-9.384
CSCuN 201	457, 519	0.013, 0.006	n.a.	-9.269
CSCuN 231	818	0.105	-12.949	-8.907
CSCuN 291	612	0.168	-13.267	-8.762
CSCuN 351	632	0.007	-12.809	-9.272
CN1CuS 9	627	0.158	-13.268	-8.780
CN1CuS 69	626	0.170	-13.248	-8.778
CN1CuS 189	640	0.143	-13.225	-8.767
CN1CuS 249	625	0.166	-13.246	-8.779
CN1CuS 309	616	0.169	-13.309	-8.788
CN2CuS 35	637	0.159	-13.205	-8.786
CN2CuS 95	627	0.173	-13.200	-8.764
CN2CuS 215	626	0.171	-13.235	-8.784
CN2CuS 275	622	0.180	-13.229	-8.781
CN2CuS 335	624	0.167	-13.256	-8.790

Perhaps expectedly, no distortion along the copper-imidazole bonds (be it rotation or elongation/compression) appears to cause dissolution of the 624-nm band – although shifts of up to ~50 nm in wavelength and ~10% in extinction coefficients are still seen, as illustrated in Table 1.

Table 2 shows DFT-derived relative energies entailed by some of the strongest distortions discussed in Table 1. It can be seen that all of these distortions are energetically feasible, costing at most 10 kcal/mol, and some less than 5 kcal/mol – all of which can easily be compensated by the protein environment. The initial structure seems to lie slightly higher in energy than two of the “distorted” ones; we interpret this as evidence for protein-induced sterical constraints on the active site geometry – unavoidable when one considers the need to fit the β carbon atoms of the aminoacid ligands, as well as the atoms connected to them.

Table 2 also shows DFT-derived partial atomic charges at the copper and at the three atoms coordinated to it do suffer well-defined changes in some of the distorted structures, both along the Cu-S and along the Cu-N distortions. In particular, the changes in spin density at the copper suggest that such distortions (some of them already shown to cause strong effects on the UV-vis spectra) would also be detectable in EPR, by way of hyperfine coupling constants. Conversely, changes in Cu-N bond lengths, which did not entail disappearance of the 624-nm band in UV-vis calculations, are shown to cause changes of up to 40% in spin density at the copper, again implying that such a distortion might be detectable with EP spectroscopy.

Table 2. DFT-derived relative energies and selected electronic structure parameters for the laccase type 1 site.

Structure	Rel E kcal/mole	Mulliken atomic charges//atomic spin densities			
		N1	N2	Cu	S
initial	+0.8	-0.09/0.04	-0.14/0.04	0.38/0.52	0.04/0.41
Cu-S 1.98	+7.7	-0.07/0.04	-0.13/0.04	0.34/0.47	0.04/0.45
Cu-S 2.38	+5.7	-0.12/0.03	-0.16/0.03	0.42/0.45	0.05/0.47
Cu-N1 1.91	+2.2	-0.08/0.05	-0.16/0.04	0.35/0.53	0.03/0.38
Cu-N1 2.41	+8.5	-0.19/0.02	-0.17/0.025	0.49/0.37	0.14/0.59
Cu-N2 2.44	+10.1	-0.12/0.03	-0.19/0.02	0.44/0.31	0.16/0.65
CSCuN 291	+0.5	-0.11/0.04	-0.11/0.04	0.37/0.50	0.05/0.42
CN1CuS 309	0.0	-0.11/0.03	-0.15/0.04	0.39/0.52	0.02/0.41
CN2CuS 335	+0.2	-0.07/-0.00	-0.14/0.04	0.34/0.52	-0.01/0.40

Table 3 shows geometrical parameters along the Cu-N and Cu-S bonds in known crystal structures of laccases. All of these structures represent “blue” laccases. It may be seen that none of these features a Cu-S bond at ~2.4-2.5 Å (a value which according to calculations would have turned it from blue to yellow), and that dihedral angles tend to be grouped at values of at most +/- 30º relative to the average.

Table 3. Relevant geometrical parameters at intact type 1 copper centers in structures of laccases found in the Protein Data Bank.

pdb ID	Resolution (Å)	Cu-S	Cu-N 1	Cu-N 2	SCu-NC(1)	SCu-NC(1)
1A65	2.33	2.28	1.91	1.87	-19	-55
1GSK	1.70	2.20	2.05	2.06	-8	-51
1GW0	2.40	2.20	1.91	1.93	-2	-70
		2.22	1.93	1.92	-1	-73
1GYC	1.90	2.19	2.02	2.04	-5	-50
1HFU	1.68	2.19	2.07	2.03	-7	-45
1KYA	2.40	2.20	2.36	2.23	9	-36
		2.25	2.36	2.17	6	-38
		2.23	2.31	2.22	7	-36
		2.31	2.35	2.14	9	-37
1OF0	2.45	2.15	2.07	2.10	-7	-46
1UVW	2.45	2.11	2.11	2.23	-33	-12
1V10	1.70	2.17	2.14	2.15	-32	-56
1W6L	2.00	2.22	2.02	1.98	-16	-32
1W6W	2.20	2.16	2.02	2.07	-17	-44
1W8E	2.20	2.21	2.01	1.99	1	-47
2BHF	2.50	2.26	1.98	2.00	-5	-54
2FQD	2.40	2.22	2.17	2.31	-22	-40
2FQE	1.92	2.18	2.06	2.05	-13	-53
2FQF	2.00	2.22	2.03	2.13	-18	-28
2FQG	2.30	2.17	2.05	2.16	-6	-35
2H5U	1.90	2.31	2.00	1.99	13	-29
2HRG	1.58	2.15	2.06	2.03	3	-47
2HRH	2.60	2.14	2.09	2.09	-44	-52
2HZH	2.60	2.11	2.53	2.37	2	-49
2IH8	2.00	2.18	1.92	1.95	3	-59
		2.18	1.95	1.94	-2	-58
2IH9	2.00	2.16	1.94	1.91	-13	-46
		2.16	1.92	1.95	-8	-64
2Q9O	1.30	2.18	1.94	2.02	-12	-52
		2.15	2.00	2.00	-19	-46
2QT6	1.50	2.23	2.03	2.01	3	-47
		2.25	2.03	2.01	0	-47
2VDS	2.30	2.12	2.35	2.49	5	-45
2VDZ	1.70	2.22	2.10	2.11	7	-56
2WSD	1.60	2.27	2.03	2.01	-1	-51
2X87	2.00	2.27	2.01	2.01	2	-49

pdb ID	Resolution (Å)	Cu-S	Cu-N 1	Cu-N 2	SCu-NC(1)	SCu-NC(1)
2X88	1.80	2.21	2.08	2.07	-5	-54
2ZWN	1.70	2.24	2.08	1.99	-5	-62
		2.25	2.10	2.06	1	-67
		2.27	2.08	2.03	-2	-66
3CG8	2.68	2.20	2.01	1.89	-28	-48
		2.16	2.01	1.91	-28	-49
		2.19	1.97	1.97	-27	-52
3DIV	1.76	2.09	2.52	2.21	29	-46
3DKH	2.40	2.19	2.16	2.28	22	-60
		2.12	2.14	2.08	9	-54
3FPX	1.80	2.24	2.01	2.05	6	-38
3FU7	1.67	2.13	2.03	2.08	-16	-50
		2.19	2.00	2.05	-15	-54
3FU8	1.80	2.06	2.10	2.11	-26	-49
		2.13	2.04	1.99	-18	-51
3FU9	2.00	2.13	1.81	2.37	-10	-54
		2.01	2.06	2.28	-22	-85
3KW7	3.44	2.13	2.14	2.05	3	-71
		2.08	2.06	2.12	-8	-73
Average		2.20	2.07	2.08	-7	-50

CONCLUSIONS

Using computational methods, torsion and elongation-type deformations have been identified, which allow a “blue” tri-coordinated type 1 copper center to apparently lose its characteristic 600-nm band responsible for its blue color both by shifting it by more than 200 nm, and, in some cases, by decreasing the extinction coefficients. However, DFT calculations suggest that such distortions might also be detectable with EPR spectroscopy. Last but not least, we note that some of the distortions, while perfectly feasible in the small models employed in the present study, may well leave the site too exposed to a fourth ligand and thus may entail a lack of stability that would preclude facile experimental observation of such a distorted center.

EXPERIMENTAL SECTION

All data reported here are single-point energy calculations; attempts to optimize the geometry at the DFT level led to significant deviations from the initial conformations of the ligands. ZINDO/S-CI calculations were performed in the Hyperchem software package [12]. An excitation window of 20 eV was chosen; a ~ 600-nm band of reasonable intensity was found to already be computed with windows as low as 14 eV, and its wavelength appeared to converge towards a stable value as the excitation window was gradually increased up to 20 eV. DFT calculations on selected structures were performed in the Gaussian09 software package [13], M062X/6-311+G** using the SCF=Tight option.

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