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U25

Steady-state Kinetics of *Rhus* Laccase with Rapid Substrates

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Rhus laccase exhibits ping-pong kinetics [1]. Though reductants do not bind, an apparent K_m reflecting substrate-independent steps was reported with a DMPD, a rapid substrate. This work examines the effects of pH, D_2O and anions on reductant substrate-dependent (k_r) and substrate-independent (k_{cat}) steps.

Activity was measured with an O_2 electrode using DMPD as the reductant. The pH dependency of k_r is bell-shaped indicating contributions from at least two groups. The group required in its dissociated form has an apparent pk_a 7.55 ± 0.12 as reported previously [1], while the group required in its undissociated form has an apparent pk_a 8.43 ± 0.23. Anaerobic reduction data does not detect pH-dependencies consistent with these pk_a values and forms [2, 3]. In particular, no group with pk_a near 7.5 required in its undissociated form is detected. Therefore, the pH-dependency of k_r must involve enzymic states specific to catalytic turnover. Both pH-dependent steps are more likely associated with type 2 Cu than type 1 Cu reduction. Type 1 Cu(II) reduction in laccase which has been activated by a reduction-reoxidation cycle does not show these pH-dependencies [4]. A recently derived steady-state rate law implies that this also holds for type 1 reduction during turnover [4].

The pH dependence of k_{cat} is also bell-shaped. The implicated pk_a values were: pk_a 5.91 ± 0.035 for an acid catalyst and pk_a 8.99 ± 0.02 for a base catalyst. Residual activity (0.22 maximal) at high pH, which implies that the putative acid catalyst is not mandatory, was accounted for in the data fits. While k_r does not show a solvent isotope effect, k_{cat} does. In 50% D_2O, pH 7.40, k_H/k_D is 1.36, in 100%, 2.12 ± 0.038. The ratio of the pH independent k_{cat} is 1.48 in 50% D_2O. Thus, proton(s) transfers are implicated in a rate-limiting substrate-independent step. Analyses of the D_2O concentration dependence of k_{cat} at pH 7.4 are consistent with 2 proton transfers. The isotope-exchanging group is most likely functioning as the acid catalyst given the pk_a of the base catalyst and the magnitude of the effect at pH 7.40.

Both F^- and N_3^- inhibit laccase immediately when they are added during steady-state turnover. The inhibition patterns obtained indicate that both F^- and N_3^- inhibit both reductant-dependent and substrate-independent steps. Laccase exhibits partial activity for both the k_r and k_{cat} effects when saturated with F^-. ESR spectra of laccase at pH 6.0, 4 °C, show that both types 1 and 2 Cu are 30% reduced during steady-state turnover. The concentration of reduced type 1 and 2 are significantly increased when 40 mM F^- is added. Stopped-flow experiments show that F^- does not affect type 3 reoxidation of O_2 binding. Thus, the ESR results indicate that during steady-state turnover in the presence of F^-, the rate-limiting step is a substrate-independent step affecting type 1 and type 2 Cu reoxidation. These results also imply that F^- can remain bound to the reduced type 2 Cu.

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U26

^{1}H-NMR Conformational Studies of Biomolecules and their Complexes with Diamagnetic Metal Ions: Solvent Exposure Delineations of Proton Nuclei by Using Stable Nitroxides

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Nitroxide induced perturbations of proton relaxation rates of compounds of established solution structure has been shown to be mainly correlated to the hydrogen solvent exposure and, hence, to the molecular conformation [1]. The solution dynamics and relaxation mechanisms involved in the nitroxide-

biomolecule interactions have been analyzed and experimental data suggest that solvent exposed amide groups are hydrogen bonded with nitroxide N-oxyl moieties. This interaction and random biomolecule–nitroxide collisions determine the extent of observed paramagnetic effects on proton relaxation rates. Dipolar relaxation occurs between the unpaired electron of the nitroxide and proton nuclei of the biomolecule, but a quantitation of relaxation enhancements in terms of exposure factors is not straightforward, due to the complexity of biosystems where many simultaneous interacting sites have to be taken into account. Nevertheless, a qualitative insight into solvent accessibility to protons attached to complex molecular systems and the conformation of their diamagnetic metal complexes is possible.

Relaxation data obtained for the 2,2,6,6-tetramethyl piperidine-1-oxyl/gramicidine S system in DMSO, shown in Table I, confirm that hydrogen bonded and/or solvent shielded protons have the smallest molar paramagnetic relaxation S_{1p}.

TABLE I. Relaxation Data.

	ppm[a]	S_{1p} (sec^{-1} M^{-1})
Leu NH	8.31	100 ± 6
Leu H	4.64	107 ± 7
Orn NH	8.62	380 ± 13
Orn H	4.94	40 ± 10
Phe NH	9.11	350 ± 15
Phe H	4.47	235 ± 10
Pro H	4.42	130 ± 6
Methyls	1.04	370 ± 14
Aromatics	7.20	410 ± 16

[a]ppm from internal TMS.

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U27

Application of Luminescence and Absorption Spectroscopy and the X-ray Method to the Study of Ln^{3+} Ions Interaction with Aminoacids

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Lanthanide ions have found increasing use as metal ion probes for spectroscopically inert Ca(II), and substitution of Ln(III) for Ca(II) enables the performance of absorption, luminescence and magnetic resonance studies on systems of biological interest.

In peptide and protein complexes, the Ca(II) ion is normally bound by aspartate and glutamate residues so it is crucial to the full understanding of Ln(III) binding by these materials.

In our previous papers we have examined the interaction of lanthanide ions with L-asparagine, L-glutamine, L-aspartic and L-glutamic acid in aqueous solutions. We have suggested the appearance of the dimeric forms in the latter case [1–4]. Now we report the results of our spectroscopic studies of the crystals of the Nd, Ho and Eu compounds with glycine and L-glutamic acid in solid state and of the X-ray investigations of the $[Nd(gly)_3][ClO_4]_3 \cdot 5H_2O$ crystal.

Several lanthanide ion complexes with glycine and L-glutamic acid were synthetized and yield in the form of monocrystals. Absorption spectra of the crystals were measured on a Cary 14 spectrophotometer at room temperature and 5 K. Luminescence spectra of Eu(III) and Nd(III) compounds were recorded at the same temperature. E_1 and E_2 selective excitation energies of the Nd(III) ion level revealed that the Nd(III) ion could appear in two different symmetry positions in the complexes with glutamic acid. From the measured absorption spectra the oscillator strengths of f–f transitions were estimated. The relation between f–f transition intensity and position of the crystallographic axis of crystals has been stated.

The f–f transitions were analysed on the basis of the Judd theory taking the dependence of intensity on the crystallographic axis position into account.

Intensity analysis of f–f transitions revealed quite a considerable difference in the intensity distribution of bands, especially for hypersensitive transitions. Drastic differences in Judd parameters have been found (Table I).

TABLE I. The τ_λ Parameters Values.

Compound		$\tau_2 \times 10^9$	$\tau_4 \times 10^9$	$\tau_6 \times 10^9$
$[Nd(Gly)_3]$-$[ClO_4]_3$·$5H_2O$	*a*	3.65 ± 0.85	5.48 ± 0.78	11.49 ± 1.10
	b	3.25 ± 0.68	5.67 ± 0.63	10.70 ± 0.88
	c	1.83 ± 0.93	6.17 ± 0.86	10.40 ± 1.20
	a (mean)	2.83 ± 0.76	5.92 ± 0.71	10.80 ± 0.99
$[Nd(Glu)][ClO_4]_2$·$7H_2O$		4.72 ± 0.73	5.44 ± 0.68	12.44 ± 0.95

The crystal structure data determined by us for $[Nd(Gly)_3][ClO_4]_3 \cdot 5H_2O$ compound with space group P1, Z = 4, symmetry for Nd^{+3}; I-1 and C.N. = 9 (Fig. 1) confirm our suggestion that in lanthanide