(c) O. Yamauchi and A. Odani, *J. Am. Chem. Soc., 103*, 391 (1981).
(d) A. Odani and O. Yamauchi, *Inorg. Chim. Acta, 66*, 163 (1982).
8 TMO was synthesized as described in [9]; the other materials were purchased from Fluka AG, Buchs, Switzerland, from Merck AG, Darmstadt, Germany, or from Sigma Chemical Co., St. Louis, USA. All constants were determined from potentiometric pH titrations; the ionic strength (I) was adjusted with $NaClO_4$; for further experimental details see, *e.g.*, [5]. Three times the standard error of the mean value for the listed constants is ≤ 0.02 log units.
9 T. Takemoto, K. Daigo and N. Takagi, *Yakugaku Zasshi, 84*, 1180 (1964).
10 H. Gampp, H. Sigel and A. D. Zuberbühler, *Inorg. Chem., 21*, 1190 (1982).
11 H. Sigel, *Angew., Internat. Ed. Engl., 21*, 389 (1982).

U25

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

G. B. KOUDELKA and M. J. ETTINGER

State University of New York, Buffalo, N.Y. 14214, U.S.A.

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.

1 L. C. Petersen and H. Degn, *Biochim. Biophys. Acta, 526*, 85 (1978).
2 B. Reinhammar and L.-E. Andréasson, *Biochim. Biophys. Acta, 445*, 579 (1976).
3 B. Reinhammar and L.-E. Andréasson, *Biochim. Biophys. Acta, 568*, 145 (1979).
4 F. B. Hansen, G. B. Koudelka, R. W. Noble and M. J. Ettinger, *J. Am. Chem. Soc.*, submitted.

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

NERI NICCOLAI, LIONELLO POGLIANI, ELDA PERICCIOLI and WILLIAM A. GIBBONS

Istituto Chimica Generale, Università di Siena, Italy

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-