ON THE PROCESS OF ENZYMATIC OXIDATION OF HYDROQUINONE

Takao Nakamura

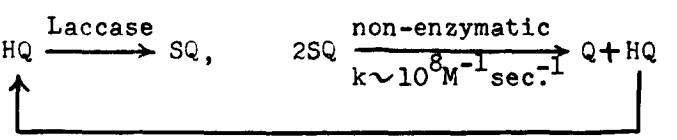
Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pennsylvania

Received January 19, 1960

It has been reported that laccase oxidizes dimethyl-p-phenylenediamine and tetramethyl-p-phenylenediamine, and this reaction gives free radicals, Wurster's red and Wurster's blue, upon transfer of one electron from these diamines to laccase copper (Nakamura and Brill, 1959). In the present study, this enzyme was also found to accelerate the oxidative formation of p-benzosemiquinone (SQ) from p-benzohydroquinone (HQ), by using a Varian EPR spectrometer with 100 kc. modulation. SQ was identified by the hyperfine structure and g-value of the spectrum. By using a flow apparatus which is connected with a capillary of 1 mm. diameter inserted into the cavity, the kinetics of SQ were followed with a fixed field strength set on the peak of the SQ spectrum. Molecular oxygen and oxidized laccase copper in the solution, although they are paramagnetic, do not interfere the SQ spectrum. All the experiments were performed at room temperature. The concentration of unpaired electrons was calculated with the calibration by 1,1-diphenyl-2-picryl hydrazyl. On mixing 10 mM HQ with pH 7.1 buffer under air-saturated conditions, 1 μM SQ was formed by autooxidation in 4 min., but in the presence of 2 μM laccase, the SQ level went up to an equilibrium value of 3 μM within 30 sec. It is known that SQ may exist in equilibrium with p-benzoquinone (Q) and HQ,

so the observation of SQ does not prove that SQ is the first product of the enzymatic reaction. Two more experiments, however, provided the proof. When 10 μM Q and 10 mM HQ were mixed in the absence of laccase, the equilibrium concentration of SQ was 0.25 μM. By the accelerated flow method, the concentration of SQ at 0.1 sec. after mixing 2 μM laccase with 10 mM HQ was 1 μM, at which time the O_2 consumed was calculated to be 1.0 μM or enough to yield 2.0 μM Q. This rapid increase of SQ on adding laccase to HQ strongly supports the hypothesis that SQ is the first product of the oxidation by laccase. There is another possibility for the SQ formation, that the reaction HQ + Q $\rightarrow$ SQ is catalyzed by laccase; but because no difference in SQ kinetics was found when laccase pre-incubated with Q was used instead of native laccase, this catalysis must be negligible.

Kinetic data from the accelerated flow experiments show that SQ produced by enzymatic oxidation decomposes with a rate which is proportional to $(SQ)^2$. The second order velocity constant of the decomposition was calculated to be about $10^8 M^{-1} sec.^{-1}$ under the present experimental conditions. The dependence of the decomposition rate on $[SQ]^2$ can be explained either by dismutation or dimerization of SQ. The stoichiometry of laccase copper-HQ reaction has been found to be 2:1 (Nakamura, 1958), which suggests dismutation is the more probable way of SQ decomposition. The proposed oxidation process is summarized in the following cyclic mechanism:



The conclusion is that HQ is oxidized to SQ by laccase, and SQ thus formed dismutates before it is further oxidized to Q by laccase. The second step of the over-all oxidation reaction

SQ→Q is a non-enzymatic process. The details of this work will be published elsewhere.

The author wishes to thank Dr. Britton Chance for his kind guidance throughout this work.

References

Nakamura, T., Biochim. Biophys. Acta, 30, 538 (1958).

Nakamura, T. and Brill, A. S., Federation Proc., 18, 292 (1959).