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^{127}I NUCLEAR MAGNETIC RESONANCE STUDIES ON THE INTERACTION OF IODIDE ION WITH HORSERADISH PEROXIDASE

Junji SAKURADA, Toichiro HOSOYA,* Toru SHIMIZU,†
and Masahiro HATANO†
Faculty of Pharmaceutical Sciences, Chiba University,
Yayoi-cho, Chiba 260
†Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira, Sendai 980

The binding of I^- to horseradish peroxidase was studied with ^{127}I NMR technique. The broadened line width of I^-/enzyme solution was further increased remarkably by decreasing pH. The change of ^{127}I NMR line width of the I^-/enzyme solution caused by decreasing pH bore resemblance to the change of iodide oxidation catalyzed by the enzyme at acidic pH.

Horseradish peroxidase (HRP) catalyzes the oxidation of iodide in the presence of H_2O_2 as well as a number of organic compounds. Thus, the enzyme has been studied as a model enzyme for thyroid peroxidase and lactoperoxidase, whose iodide oxidation is essential for their physiological roles, by using various physicochemical methods.[1-3] However, detailed mechanism on the electron transfer reaction remains unclear. Recently, the nuclear magnetic resonance of halogen ions were found to provide a useful probe for exploring the interaction of halogen ions and macromolecules[4-9] and the chloride probe technique was in fact applied to the binding to HRP[5] and chloroperoxidase.[9] The half-band width ($\Delta\nu_{1/2}$) of iodide ion, however, is very broad (usually more than 1 kHz) and only few reports applying the iodide probe to macromolecules have been published.[10,11] The present paper reports that the iodide NMR probe technique is usable, in spite of the broad half-band width, for detecting the binding of I^- to HRP, providing some suggestions on the electron transfer reaction. To our knowledge, this is the first ^{127}I NMR study applied to enzyme systems.

^{127}I NMR spectra were accumulated on a Bruker CXP-300 FT NMR spectrometer at 60.034 MHz in a 10 mm sample tube with internal D_2O for the frequency lock. Other experimental conditions were essentially the same as described previously.[12] Horseradish peroxidase (isozyme C) was obtained from Toyobo Co. The RZ(OD_{403}/OD_{280}) was 3.0. The rate of oxidation of I^- by the enzyme was

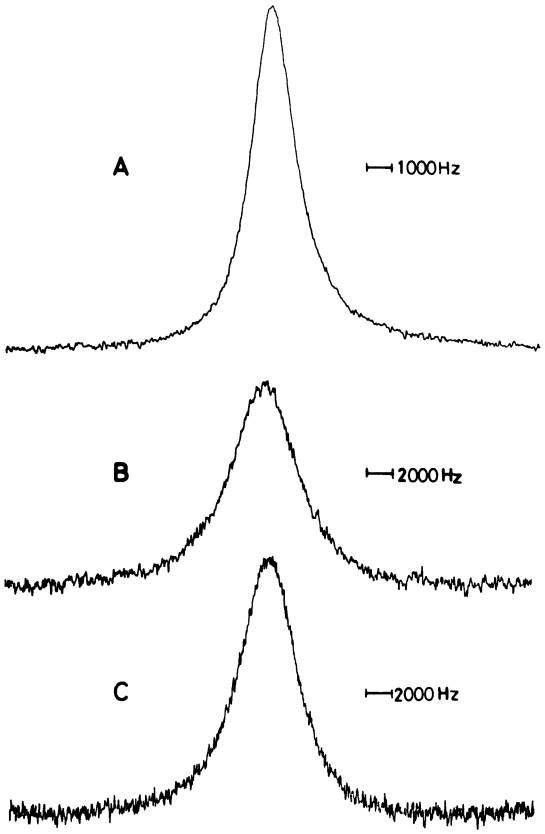

Fig. 1. ^{127}I NMR spectra of (A) KI (50 mmol dm^{-3}) at pH 4.5; (B) KI (50 mmol dm^{-3}) and horseradish peroxidase (80 μmol dm^{-3}) at pH 5.4; (C) KI (50 mmol dm^{-3}) and horseradish peroxidase (80 μmol dm^{-3}) at pH7.0 in 20 mmol dm^{-3} citrate/20 mmol dm^{-3} phosphate buffer. Temperature was kept at 290 ± 0.5 K.

measured under the conditions described previously except pH.[13]

Typical ^{127}I NMR spectra are shown in Fig. 1. An aqueous KI solution (50 mmol dm^{-3}, pH 4.5) had a very broad ^{127}I NMR signal having a $\Delta\nu_{1/2}$ of approximately 2.0 kHz (Fig. 1A). The $\Delta\nu_{1/2}$ of KI was hardly changed by changing pH from 2.4 to 7.0 (closed circles in Fig. 3). The $\Delta\nu_{1/2}$ of KI solution (50 mmol dm^{-3}) was, however, markedly broadened by adding HRP, becoming 5.9 kHz at pH 5.4 (Fig. 1B) and 5.0 kHz at pH 7.0 (Fig. 1C). Figure 2 shows that the $\Delta\nu_{1/2}$ of the I^-/HRP solution changed linearly with the concentration of enzyme. As shown in Fig. 3 (open circles), the $\Delta\nu_{1/2}$ decreased significantly with increasing pH, approaching to 5.0 kHz at pH 6-7. It is noteworthy that the curve describing the pH dependent change of $\Delta\nu_{1/2}$ in the I^-/HRP solution is apparently correlated with the development of enzyme activity (crosses in Fig. 3). Figure 3 also shows that the $\Delta\nu_{1/2}$ of I^- in the acidic region is slightly reduced by the high concentration of Cl^- but effectively reduced by the low concentration of CN^-. The CN^- and Cl^- ions are known to bind to the heme iron under the conditions used.[14,15]

The data obtained here suggest that there are at least two types of I^- binding to HRP. In the pH region above pH 6.0, ^{127}I NMR line broadening caused by the enzyme is relatively small. The small line-broadening at pH 6.0-7.0

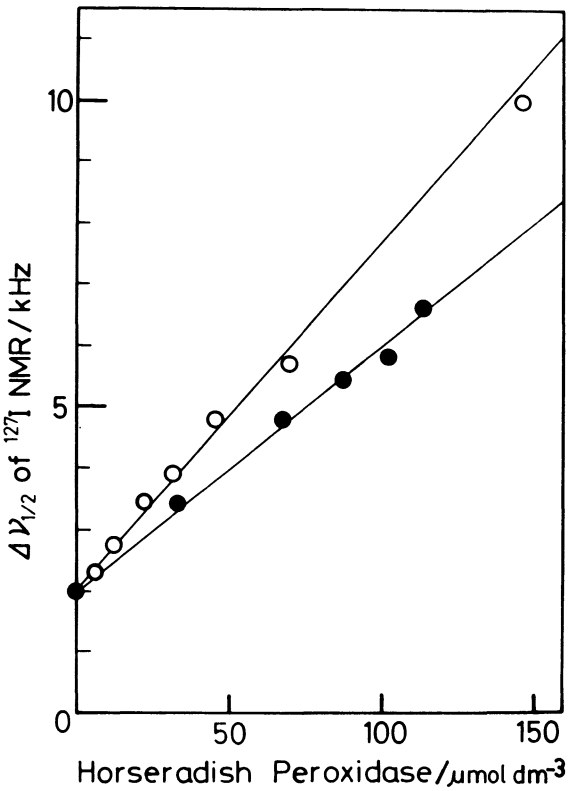

Fig. 2. Changes of ^{127}I NMR line width for KI (50 mmol dm^{-3}) (○) (pH 4.5) and for KI (50 mmol dm^{-3}) (●) (pH 7.0) caused by adding horseradish peroxidase in 20 mmol dm^{-3} citrate/20 mmol dm^{-3} phosphate buffer.

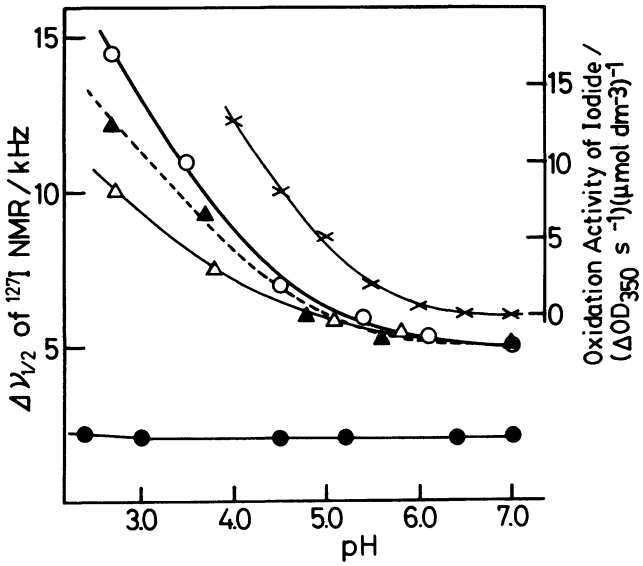

Fig. 3. ^{127}I NMR line width of I^- and the iodide oxidation activities (×) of horseradish peroxidase as functions of pH. The solutions for NMR experiments consisted of KI (50 mmol dm^{-3}) (●); KI (50 mmol dm^{-3}) and the enzyme (80 μmol dm^{-3}) (○); KI (50 mmol dm^{-3}), the enzyme (80 μmol dm^{-3}) and KCN (3.8 mmol dm^{-3}) (△); KI (50 mmol dm^{-3}), the enzyme (80 μmol dm^{-3}) and KCl (100 mmol dm^{-3}) (▲) in 20 mmol dm^{-3} citrate/20 mmol dm^{-3} phosphate buffer. The rate of oxidation of iodide (×) was measured under the condition described previously.[13] The pH values were adjusted with phosphoric acid.

may be due to the nonspecific interaction between I^- and the enzyme. The nonspecific iodide binding is not associated with the enzyme activity because practically no enzyme activity was found at pH 6.0-7.0. On the other hand, in the acidic region, additional strong I^-/enzyme interaction was found. It seems likely that the line-broadening at acidic pH is associated with the iodide oxidation exerted by HRP. The similar changes of $\Delta\nu_{1/2}$ of ^{35}Cl NMR at acidic pH is already obtained in the case of Cl^-/chloroperoxidase solution,[9] which catalyzes the oxidation of Cl^-. Absorption spectral studies indicate that I^- does not coordinate to the sixth position of the heme iron in HRP while Cl^- and CN^- do under the conditions studied here.[16] The result that Cl^- hardly influences the increase in $\Delta\nu_{1/2}$ of ^{127}I NMR in the acidic region (Fig. 3) thus

suggests that a protonation on the protein moiety or the heme ring generates the potential binding site for I^-. The view is in accordance with the data obtained by steady-state kinetic study indicating that Cl^- inhibits the I^- oxidation in the acidic region not competitively but non-competitively with I^-.[16] On the other hand, CN^- at a much lower concentration than that of Cl^- reduced the line-width in the acidic region (Fig. 3). This raises the possibility that CN^- may attack directly the acid-generated binding site to I^- or that CN^- binding at the sixth position of the heme iron may affect the binding of I^- to the acid-generated bindig site. Kinetic studies with a stopped flow apparatus are under way to help for clarifing this problem. At any rate, the broadening of the line width in acidic region is considered to reflect the association of I^- to the acid-generated binding site of HRP which is presumably prerequisite for the oxidation of I^- in the presence of H_2O_2.

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