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The Chemiluminescence of Luminol/H_2O_2 with the Ferriprotoporphyrin IX/*N*-Vinylpyrrolidone Copolymer as a Catalyst in the Neutral pH Solution

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The copolymer of ferriprotoporphyrin IX and *N*-vinylpyrrolidone, having peroxidase activity, was prepared by radical polymerization for application as a catalyst of the titled chemiluminescence. This copolymer acted as an efficient catalyst in the production of light emission sufficient to be detected in a neutral aqueous system.

Ferriprotoporphyrin IX(hemin), which is the prosthetic group of peroxidase, is a very efficient catalyst for the chemiluminescence(CL) of 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol)/hydrogen peroxide(H_2O_2) system.1) It is to be expected for the application of the CL with hemin to the highly sensitive detection of H_2O_2 in analytical and clinical chemistry. Free hemin is, however, insoluble in neutral aqueous system. Therefore, its use is restricted only to alkaline aqueous system.2) To raise the solubility of hemin, an attempt has been made to modify the water-soluble polymer. Takahashi et al. modified the hemin to monomethoxypolyethylene glycol so as to be soluble in water. As a result, peroxidase activity in the *o*-phenylene diamine/H_2O_2 system appeared not only in water but also in organic solvents.3)

Protoporphyrin IX (iron free hemin), having two vinyl groups in its molecule, is capable of forming poly(protoporphyrin IX) by vinyl polymerization.4) The vinyl group of hemin also seems to be able to use the formation of the polyvinyl compounds. In this study, the author prepared the hemin/*N*-vinylpyrrolidone(VP) copolymer so as to increase the solubility of hemin in the neutral pH solution. And the capability of this copolymer as a CL catalyst was evaluated in a neutral pH solution in which CL with a general catalyst, such as potassium hexacyanoferrate(III), is extremely weak.5)

The hemin/VP copolymer was obtained by radical polymerization at 75 °C for 24 h using azobisisobutyronitrile(AIBN) as the initiator. The polymerization mixture was placed in an ample, which was sealed under vacuum. VP and *N*,*N*-dimethylformamide(DMF) as the solvent were purified in the usual way prior to polymerization by vacuum distillation.6,7) The quantities of hemin, VP, AIBN, and DMF in an ample were 30 mg(0.057 mol%), 8.9 g(99.5 mol%), 59 mg(0.446 mol%), and 2 cm^3, respectively. The product obtained in the ample was a dark brown-colored highly viscous fluid. The product was purified by dissolving it in methanol and

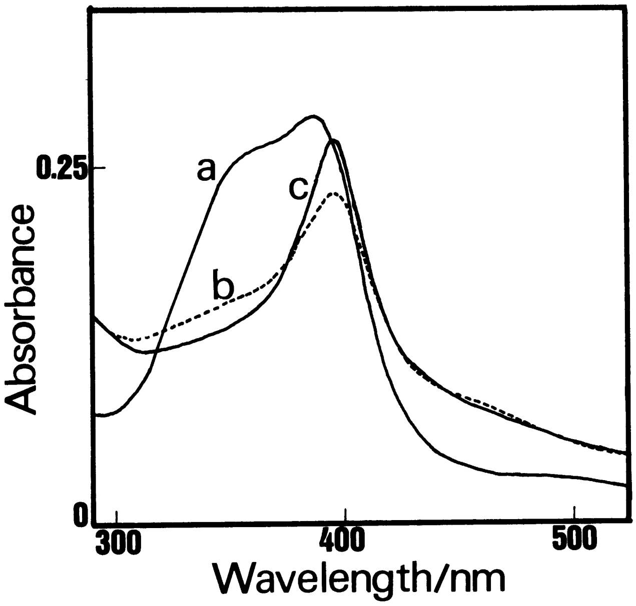

Fig. 1. Absorption spectra of the hemin/VP copolymer and hemin.

a: [hemin] = 5.0 x 10^{-5} mol dm^{-3} in 0.1 mol dm^{-3} Na_2CO_3.

b: [hemin/VP copolymer] = 0.1 wt% in 0.1 mol dm^{-3} Na_2CO_3.

c: [hemin/VP copolymer] = 0.1 wt% in phosphate buffer solution (0.1 mol dm^{-3}, pH 7.0).

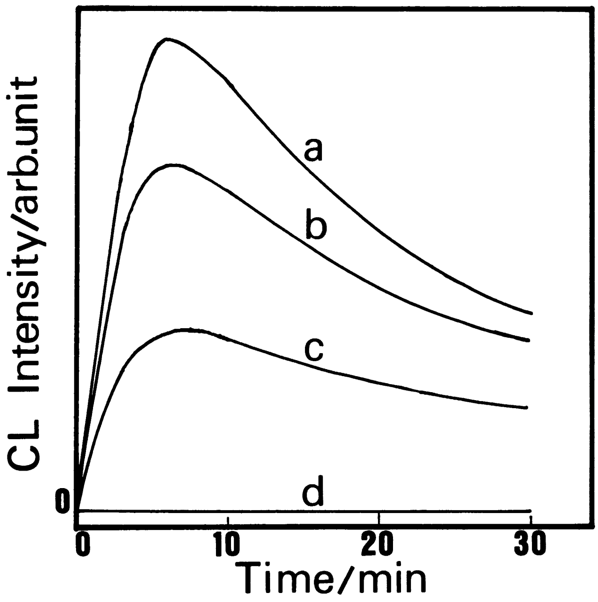

Fig. 2. Time course of CL in 0.1 mol dm^{-3}, pH 7.0, phosphate buffer solution. [luminol] = 2.44 x 10^{-4} mol dm^{-3}, [H_2O_2] = 4.30 x 10^{-5} mol dm^{-3}, [hemin/VP copolymer]: a=0.1 wt%, b=0.05 wt%, c=0.02 wt%, d=0.0 wt%.

then precipitating it in diethyl ether. The purification operation was repeated five times. The yield was about 40%. The final state of the copolymer was solid.

The average molecular weight of the copolymer was determined by gel permeation chromatography(column: Toyo Soda TSKgel G 3000PW + GP 5000PW, eluent: acetonitrile/acetate buffer solution(0.1 mol dm^{-3}, pH 5.0) = 20/80(V/V), detection: UV(230 nm) and RI). The experimental data are summarized in Table 1.

Table 1. Properties of the copolymer

	$\overline{M}w$[a]	$\overline{M}n$[b]	U[c]	Color
Hemin/VP copolymer	31500	6900	4.6	dark brown

a) The weight-average molecular weight.
b) The number-average molecular weight.
c) A measure of the polydispersity($U=\overline{M}w/\overline{M}n$).

The present hemin/VP copolymer seemed to be a many branched copolymer because the U value was larger than 2. The iron content determined by atomic absorption analysis was about 0.8 wt% / copolymer(instrument: Japan Jarrelash AA-8200, method: standard addition). This value indicated that virtually all the monomer hemin was reacted to form the copolymer because the yield was about 40%. Moreover, it was

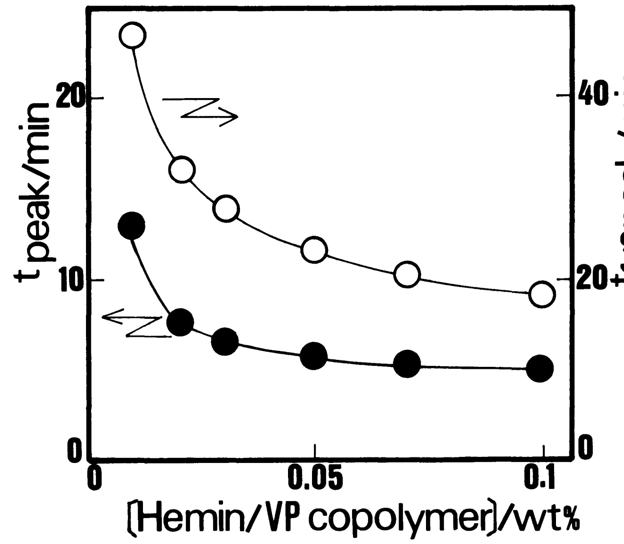

Fig. 3. Effect of the hemin/VP copolymer on t_{peak} and $t_{1/2\ peak}$.
[luminol] = 2.44×10^{-4} mol dm^{-3}, [H_2O_2] = 4.30×10^{-5} mol dm^{-3}.

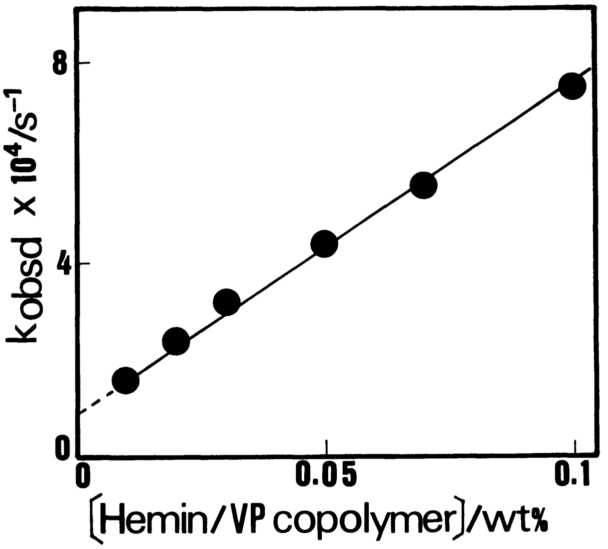

Fig. 4. Relationship between k_{obsd} and [hemin/VP copolymer].
[luminol] = 2.44×10^{-4} mol dm^{-3}, [H_2O_2] = 4.30×10^{-5} mol dm^{-3}.

possible to control the iron content, i.e. the hemin content, to some extent by varying the monomer compositions.

Figure 1 shows the absorption spectra of the copolymer and hemin(instrument: JASCO UVDEC-610C spectrophotometer). A sharp Soret band was observed at 394 nm in the absorption spectra of the hemin/VP copolymer. It shifted about 10 nm to longer wave lengths compared with the Soret band of hemin.

The evaluation of the hemin/VP copolymer as a CL catalyst was performed in phosphate buffer solution(0.1 mol dm^{-3}, pH 7.0). The CL reaction was initiated as described below. To a stirred solution(4 cm^3) of luminol containing the hemin/VP copolymer was added 0.1 cm^3 of various concentrations of H_2O_2 in a black box. The injection of H_2O_2 was done by the use of a microsyringe, and stirring was performed with a magnetic stirrer. Stirring was stopped 10 s after the injection of H_2O_2. Both solution of luminol and of H_2O_2 were prepared with phosphate buffer solution(0.1 mol dm^{-3}, pH 7.0), respectively. Light emission was received by a Hamamatsu Photonics Photomultiplier R1104, and the phototube current was recorded by a Matsushita VP-6431B recorder after amplified by a Saimaru Tsusho preamplifier. The luminol concentration(concn) before the CL reaction was kept at 2.44×10^{-4} mol dm^{-3} in all reaction systems.

Figure 2 indicates the time course of CL. In the presence of the hemin/VP copolymer, the light emission markedly increased compared with the CL in the absence of it. The CL of luminol/H_2O_2 with Fe^{3+}, Cu^{2+}, or Co^{2+} was also very weak under condition of neutral pH.

Figure 3 shows the time required to reach the CL peak(t_{peak}) and that required to decrease from the CL peak to 50% of the peak($t_{1/2\ peak}$) measured in the presence of the hemin/VP copolymer. As can be seen from Fig. 3, both times decreased with higher copolymer concn. This indicates that the hemin/VP copolymer is effective in increasing the reaction rate for the light production. When H_2O_2 concn was smaller than 4.30 x 10^{-5} mol dm^{-3}, the first-order rate analysis of the decay of the CL intensity(I) with time until about 50% CL peak afforded the observed rate constant(k_{obsd}) at a given hemin/VP copolymer concn, i.e. the pseudo-first-order rate constant. The relation between ln I and k_{obsd} is given by

$$-\ln I = A + k_{obsd}t, \qquad (1)$$

where A is a constant. In the absence of the hemin/VP copolymer, the k_{obsd} value was ca. zero.

In Fig. 4 are plotted k_{obsd} vs. hemin/VP copolymer concn. Since a linear relationship exists between k_{obsd} and hemin/VP copolymer concn, the CL reaction proceeds catalytically with this copolymer. It is under investigation that the intercept given by extrapolating the plots of k_{obsd} to zero concn of the hemin/VP copolymer has the positive value, although the measured k_{obsd} in the absence of the hemin/VP copolymer is ca. zero. On the other hand, the improvement of the catalitic efficiency given by the slope in Fig. 4 is possible to vary the monomer compositions and/or the kinds of the water-soluble vinyl monomers.

Further details of the CL with the copolymer contained hemin will be shortly reported together with the result of its analytical application elsewhere.

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