

Metabolism of Pyrogallol to Purpurogallin by Human Erythrocytic Hemoglobin

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MIYAZAKI, K., ARAI, S., IWAMOTO, T., TAKASAKI, M. and TOMODA, A. *Metabolism of Pyrogallol to Purpurogallin by Human Erythrocytic Hemoglobin*. Tohoku J. Exp. Med., 2004, **203** (4), 319-330 — The aim of this study was to investigate the oxidoreductive reactions of human hemoglobin with pyrogallol and the metabolism of pyrogallol by the protein, which contains a protoporphyrin IX like cytochrome P-450. Pyrogallol, having three hydroxy groups at the adjacent positions in the benzene ring, oxidized human oxyhemoglobin to methemoglobin and reduced human methemoglobin to oxyhemoglobin. Since superoxide dismutase and catalase inhibited these reactions extensively, active oxygens such as superoxide and hydrogen peroxide were considered to be involved in the oxidoreductive reaction of human hemoglobin by pyrogallol. It was also found that the metabolism of pyrogallol to purpurogallin occurred quickly in human erythrocytes, *i.e.*, when pyrogallol was added to human erythrocyte suspension, it oxidized intracellular hemoglobin and produced purpurogallin. The metabolism of pyrogallol to purpurogallin was explained by the pyrogallol oxidation with superoxide and hydrogen peroxide produced during the oxidoreductive reactions of human hemoglobin with pyrogallol. The present results show that human erythrocytes can metabolize pyrogallol, suggesting that the cells may be involved in the metabolism of some drugs in the human body. ——— erythrocytes; hemoglobin; pyrogallol; purpurogallin

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Though drug metabolism has been shown to be carried out by cytochrome P-450 in the liver (Omura and Sato 1964; Lu et al. 1972), the contribution of erythrocytic hemoglobin, a protoporphyrin IX containing hemoprotein like cytochrome P-450, to drug metabolism is un-

likely to attract much attention. Tomoda et al. (1977a) showed that aniline was metabolized to *p*-aminophenol by human erythrocytes, in the presence of catalytic amounts of methylene blue. Mieyal and Blumer (1976) demonstrated that oxyhemoglobin can hydroxylate aniline to *p*-ami-

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nophenol in the presence of NADPH and P-450 reductase. Monooxygenase activity of human methemoglobin in aniline hydroxylation was also demonstrated by Ferraiolo et al. (1984). Shiga and Imaizumi (1973, 1975) indicated the production of the intermediate phenoxy radical during the one-electron oxidation of organic phenol compounds caused by methemoglobin-H₂O₂ complex. The role of hemoglobin as a frustrated oxidase to metabolize some drugs was suggested by Carrell and Winterbourn (1977). Later, Tomoda et al. (1984, 1986a and 1992) found that 3-hydroxy-anthranilic acid, *o*-aminophenol, and 2-amino-5-methylphenol were metabolized to phenoxazine compounds in the presence of human hemoglobin or human erythrocytes, coupled with the oxido-reductive reactions of hemoglobin. The phenoxazine produced by the reaction of human or bovine hemoglobin with 2-amino-5-methylphenol has been shown to exert anti-cancer effects (Mori et al. 2000; Koshibu-Koizumi et al. 2002), immunosuppressive effects (Gao et al. 2002), and antiviral effects (Iwata et al. 2003).

On the other hand, pyrogallol has been used for staining leather in the leather industry and for treatment of psoriasis, however detailed studies on the biological effect of pyrogallol, especially the oxidative effect of this compound on human hemoglobin has not been clarified. Furthermore, purpurogallin was shown to be synthesized by chemical oxidation of pyrogallol (Gao et al. 1998), and to exert biological effects such as hydroxyl radical-scavenging property (Prasad and Laxdal 1994), protection of cardiovascular cells (Wu et al. 1996) and protection of erythrocyte against lysis by peroxy radicals (Sugiyama et al. 1993). However, the biological synthesis of this compound has not been demonstrated. During the study of oxido-reductive reaction of hemoglobin, we found that pyrogallol oxidizes oxyhemoglobin and reduces methemoglobin. Tomoda et al. (1984, 1986a and 1992) found that *o*-aminophenol and its derivatives can oxidize oxyhemoglobin and reduce methemoglobin, and these compounds are metabolized to phenoxazines during the oxido-

reductive reactions of hemoglobin, which was enabled by the fact that the amino group and the hydroxy group are adjacently present in the benzene ring. Since pyrogallol has three hydroxy groups at the adjacent positions in the benzene ring, it is conceivable that pyrogallol is metabolized to some tricyclic chromophore such as dibenzodioxine by the oxido-reductive reactions of hemoglobin. These facts prompted us to study whether pyrogallol is metabolized by human hemoglobin and erythrocytes. Consequently, we show that pyrogallol is rapidly metabolized to purpurogallin, without formation of the tricyclic chromophore. The present manuscript appears to be the first report on biological metabolism of pyrogallol to purpurogallin by human erythrocytic hemoglobin, and the mechanism for the oxido-reductive reactions of human hemoglobin by pyrogallol.

MATERIALS AND METHODS

Materials

Human erythrocytes were obtained after removal of plasma and buffy coats from freshly drawn heparinized blood. Then, the cells were washed twice with 0.9% NaCl solution, by centrifuging at 10 000 *g*×1 minutes. Erythrocytes thus obtained were divided into two parts: one for the experiment of reaction of crude hemoglobin or purified hemoglobin with pyrogallol, and the remaining part for the experiment of reaction of human erythrocytes with pyrogallol.

Pyrogallol and purpurogallin were purchased from Wako Pure Chemicals (Tokyo) and Sigma (St. Louis, MO, USA), respectively. Bis-Tris was purchased from Sigma. Catalase was obtained from Roche Applied Sciences (Basel, Switzerland), and superoxide dismutase and myo-inositol hexaphosphate (P₆-inositol) were obtained from Sigma. Sephadex G-25 (fine), Sephadex LH-20 and CM Sephadex C-50 were purchased from Amersham Biociences (Piscataway, NJ, USA). Silica gel plates for thin layer chromatography (HPTLC plate, 10×10 cm) were purchased from Merck (Darmstadt, Germany).

Preparation of crude and purified hemoglobin

Washed erythrocytes were lysed by adding 10-fold distilled water to the cells. After 15 minutes, the lysates were centrifuged at 10 000 $g \times 20$ minutes, to remove erythrocyte ghosts. The supernatant was designated as crude hemoglobin. The crude hemoglobin was further purified, by column chromatography using Sephadex G-25 (fine) and CM Sephadex C-50 (Tomoda et al. 1986b). This hemoglobin was designated oxyhemoglobin. Purified oxyhemoglobin was passed through a column of Sephadex G-25 (fine) previously equilibrated with 0.05 M bis-Tris buffer (pH 7.0) with 0.1 M NaCl. Purified methemoglobin was prepared by the oxidation of purified oxyhemoglobin with ferricyanide, and by passing the reaction mixture through a column of Sephadex G-25 (fine) previously equilibrated with 0.05 M bis-Tris buffer (pH 7.0) with 0.1 M NaCl.

Oxido-reductive reaction of purified human hemoglobin with pyrogallol or with purpurogallin

Twenty μl of 100 mM pyrogallol (12.6 mg/ml H_2O) solution, which was prepared just before use, was added to 2 ml solution of purified human oxyhemoglobin (50 μM in heme), or methemoglobin (50 μM in heme), to which superoxide dismutase (29 units), catalase (1300 units), superoxide dismutase (29 units) plus catalase (1300 units) or P_6 -inositol (500 μM) was previously added. The oxidation of oxyhemoglobin by pyrogallol was examined by a Hitachi 2000 spectrophotometer (Hitachi Co., Ltd., Tokyo), pursuing the decrease in absorbance at 578 nm. The reduction of methemoglobin by the pyrogallol was pursued by the increase in absorbance at 578 nm. The oxido-reductive reactions of hemoglobin by purpurogallin were spectrophotometrically pursued at 578 nm, after the addition of 20 μl of 100 mM purpurogallin (2.2 mg in 0.1 ml dimethyl sulfoxide) to 2 ml solution of oxyhemoglobin or methemoglobin, in the place of pyrogallol. Every oxidation rate and reduction rate was calculated by dividing the optical density change at 578 nm/minute with 15.3, the millimolar extinction of

oxyhemoglobin at 578 nm (Van Assendelft and Zijlstra 1975). The experiments under anaerobic conditions were performed in a Thunberg type quartz-cell, as described previously (Tomoda et al. 1986b). The oxidation of deoxyhemoglobin was spectrophotometrically pursued at 630 nm at 25°C.

Oxidation of intracellular hemoglobin by pyrogallol and metabolism of pyrogallol by human erythrocytes

Washed erythrocytes were suspended in 0.9 % NaCl solution to make the hematocrit value 15%, and were preincubated at 37°C for 5 minutes. Then, 50 mM pyrogallol solution prepared just before use, was added drop by drop to 40 ml erythrocyte suspension to make a final concentration of 5 mM. The cell suspension was incubated at 37°C for 2 hours. For the analysis of oxidation of erythrocytic hemoglobin by pyrogallol, 50 μl samples were taken out at constant intervals and then added in 3 ml ice-cold distilled water. Each hemolysate was applied to a Sephadex G-25 (fine) column (0.5 cm \times 8 cm) previously equilibrated with 20 mM potassium phosphate buffer (pH 7.0), and the red colored portion including hemoglobin was collected. This eluate was diluted with 20 mM potassium phosphate buffer to reach a final volume of 2 ml and was subjected to measurement of absorption spectra between 500 nm and 650 nm.

For the analysis of the metabolite of pyrogallol during the reaction with human erythrocytes, 2-ml sample each was taken out from the erythrocyte suspension at constant intervals (10 minutes, 30 minutes, 60 minutes and 120 minutes after the addition of pyrogallol to erythrocyte suspension), and was immediately centrifuged at 10 000 $g \times 1$ minute. The supernatant (0.5 ml) was diluted with 1.5 ml methanol and was centrifuged at 10 000 $g \times 15$ minutes to remove the denatured proteins. Then, the supernatant was subjected to the measurement of absorption spectra between 200 nm and 600 nm. The sample obtained at 10 minutes was also applied to a thin layer chromat-

ography.

In order to isolate the metabolites of pyrogallol in human erythrocytes, 10 ml sample at 10 minutes after the addition of pyrogallol was separately collected from the erythrocyte suspension and was immediately centrifuged at 10 000 $g \times 1$ minute. Five ml supernatant was collected and applied to a Sephadex LH-20 column (2 cm \times 20 cm) previously equilibrated with 50% ethanol, and then were eluted with 50% ethanol. About 10 ml solution of the main band with an orange color was collected and evaporated to the powder. Ten mg powder was used for analysis of the chemical structure of the compound as described below.

Examination of physical properties of the metabolite of pyrogallol

We examined the physical properties of the orange-colored metabolite of pyrogallol, which was produced during the reaction with human erythrocytes. UV and IR spectra were recorded on a Hitachi 2000 double beam spectrophotometer and JASCO IR-700 spectrometer, respectively. ^1H and ^{13}C -NMR spectra were acquired at 300 MHz and 76 MHz on JEOL JNM-ECP 300 FTNMR spectrometer, respectively. The chemical shifts (δ) are listed in ppm against tetramethylsilane as an internal standard, with the following abbreviations of multiplicity used; s=singlet, d=doublet. APCI-mass spectrum was obtained with a Hitachi M-1001 spectrometer. High-resolution mass spectrum (FAB, negative, m-nitrobenzylalcohol as a matrix) was recorded using a JEOL MS 700 mass spectrometer.

Metabolism of pyrogallol by crude oxyhemoglobin

One hundred μl of 100 mM pyrogallol solution, which was prepared just before use, was added to 2 ml solution of crude oxyhemoglobin (2.4 mM in heme), which was previously incubated at 37°C for 10 minutes. The reaction mixture was incubated at 37°C for 30 minutes, and then was poured into 6 ml methanol in a glass tube. After mixing well, the sample was centrifuged at

10 000 $\times g$ for 15 minutes to remove the denatured hemoglobin. The supernatant was applied to a thin layer chromatography.

Thin layer chromatography of the pyrogallol metabolites

Thin layer chromatography of the sample was performed in ascending systems (chloroform/acetone/methanol, 1 : 2 : 1, by vol.) using a silica gel plate.

RESULTS

Figs. 1 show the changes in absorbance at 578 nm during the oxidation of human oxyhemoglobin or the reduction of human methemoglobin by pyrogallol, in the presence or absence of catalase, superoxide dismutase and P_6 -inositol. Human oxyhemoglobin was oxidized by pyrogallol, and the oxidation rate was much accelerated by P_6 -inositol, but was extensively inhibited by superoxide dismutase, catalase or superoxide dismutase plus catalase (Fig. 1 A). The upper column of Table 1 shows the oxidation rate of oxyhemoglobin by pyrogallol. Consequently, the inhibition of hemoglobin oxidation rate was 72%, 72%, and 89% of the control, in the presence of superoxide dismutase, catalase and superoxide dismutase plus catalase, respectively, indicating that the contribution of superoxide and hydrogen peroxide, which are produced during the reaction of oxyhemoglobin with pyrogallol, to the oxidation of oxyhemoglobin with pyrogallol is predominant. We also found that deoxyhemoglobin was not oxidized by pyrogallol (data not shown), suggesting that oxygen is necessary for the oxidation of hemoglobin by pyrogallol, and being consistent with major contribution of active oxygens such as superoxide and hydrogen peroxide to the reaction.

The reduction of human methemoglobin was caused by pyrogallol (Fig. 1B). The rate of reduction of methemoglobin was comparable to that of oxidation of oxyhemoglobin, and was much accelerated in the presence of P_6 -inositol. However, it was extensively suppressed in the presence of superoxide dismutase, catalase or superoxide dis-

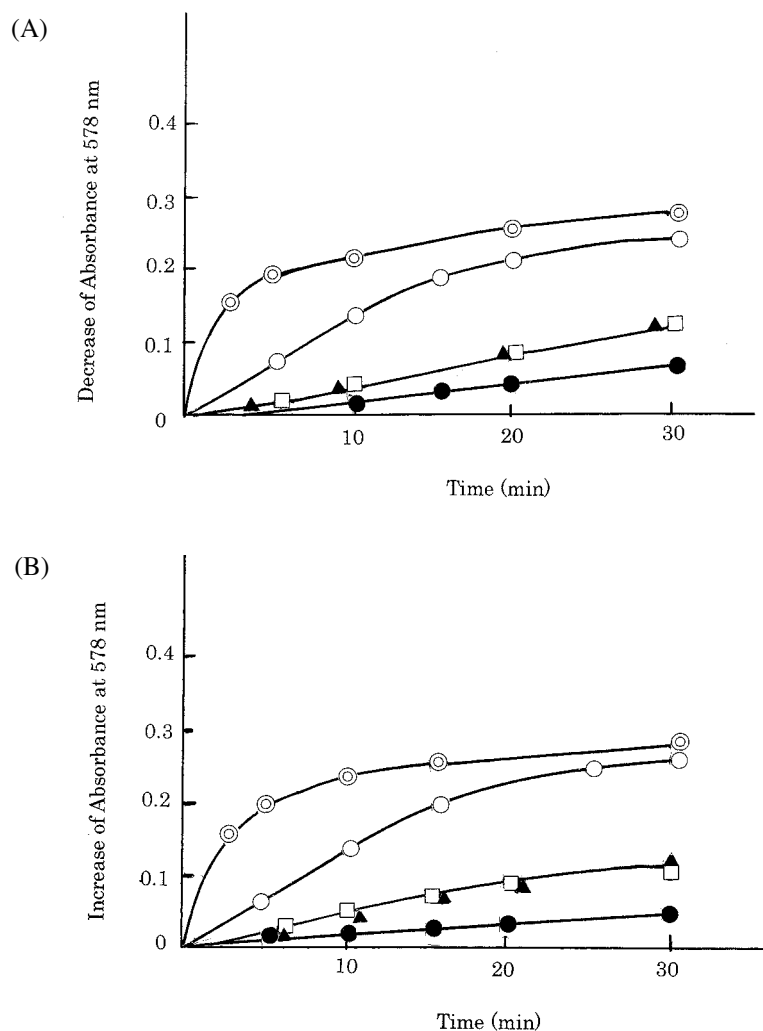


Fig. 1. Oxido-reductive reactions of purified human hemoglobin with pyrogallol.

(A). Oxidation of oxyhemoglobin by pyrogallol in the presence or absence of superoxide dismutase (SOD), catalase, or P₆-inositol. (B). Reduction of methemoglobin by pyrogallol in the presence or absence of catalase, superoxide dismutase, or P₆-inositol. ○, control □, + catalase; ▲, + SOD; ●, SOD plus catalase; ⊙, + P₆-inositol.

mutase plus catalase (the lower column of Table I). The inhibition of methemoglobin reduction rate was 65%, 72% and 86% of the control, in the presence of superoxide dismutase, catalase and superoxide dismutase plus catalase, respectively, indicating that the active oxygens such as superoxide and hydrogen peroxide play a role in the reduction of methemoglobin by pyrogallol, and that the reduction rate of methemoglobin in the presence of superoxide plus catalase (14% of the

control) may be considered as the genuine reduction of methemoglobin by pyrogallol.

Fig. 2 shows the changes in absorption spectra of the erythrocytic hemoglobin between 500 nm and 650 nm, after the addition of pyrogallol to human erythrocyte suspension. The absorption spectra changed with time, accompanying the increase in absorbance at 630 nm and decrease in absorbance at 578 nm with isobestic points at 522 nm and 586 nm, suggesting that intracellular

TABLE 1. *The rate of oxido-reductive reactions of human hemoglobin with pyrogallol*

Oxidation rate of oxyhemoglobin by pyrogallol ($\mu\text{M heme}/\text{min}$)	
Control	0.85
+ catalase	0.25
+ superoxide dismutase	0.25
+ catalase, superoxide dismutase	0.1
+ P ₆ -inositol	7.86
Reduction rate of methemoglobin by pyrogallol ($\mu\text{M heme}/\text{min}$)	
Control	0.92
+ catalase	0.33
+ superoxide dismutase	0.26
+ catalase, superoxide dismutase	0.13
+ P ₆ -inositol	6.5

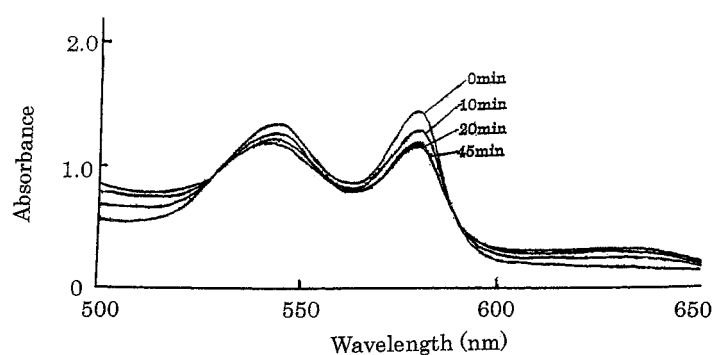


Fig. 2. Oxidation of intracellular hemoglobin in human erythrocytes by pyrogallol. The changes in absorption spectra of human erythrocytic hemoglobin caused by the addition of pyrogallol to human erythrocytes were measured between 500 nm and 650 nm.

hemoglobin was oxidized to methemoglobin by pyrogallol. By further incubation of erythrocytes with pyrogallol, the absorption spectra moved upward, shifting out of the isobestic points (data not shown), suggesting that denaturation of hemoglobin began in the cells.

We studied the metabolism of pyrogallol by human erythrocytes. Fig. 3 shows the changes in absorption spectra of the supernatant of the erythrocyte suspension collected at 10 minutes, 30 minutes, 60 minutes and 120 minutes after the addition of pyrogallol. The absorption spectra of the supernatant obtained at 10 minutes were quite different from those of authentic pyrogallol (0 minute) and almost agreed with those of authentic purpurogallin with a maximal absorbance at 320

nm in 10 mM potassium phosphate solution (pH 7.0). Since the millimolar extinction coefficient of purpurogallin was estimated to be 32.2 at 320 nm in 10 mM potassium phosphate solution (pH 7.0), purpurogallin in the erythrocyte suspension obtained at 10 minutes could be estimated 164 μM .

The absorption spectra of the sample at 30 minutes showed the decrease in absorbance at 320 nm without the change in absorbance at 400 nm. The sample at 60 minutes was characteristic for its absorptional peak at 400 nm, which disappeared in the sample at 120 nm.

In order to identify the metabolic substances, we purified the substance in the sample obtained at 10 minutes by using a column of Sephadex LH-20. There were two main bands including the

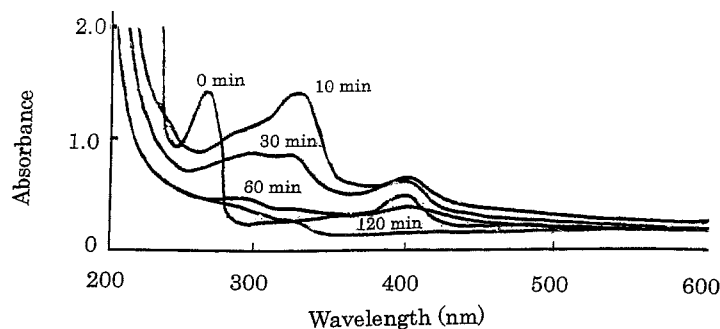


Fig. 3. Metabolism of pyrogallol in human erythrocytes. The changes in absorption spectra of the supernatant obtained after the addition of pyrogallol to human erythrocyte suspensions, were measured between 250 nm and 600 nm. The absorption spectra of 0 min sample show those of 1 mM pyrogallol in 10 mM potassium phosphate solution (pH 7.0).

faster brown band and the slower orange band on column chromatography. We collected the eluate containing the orange band, and evaporated it to powder. The powder was subjected to measurement of NMR spectra. The results are summarized in Table 2. Consequently, the APCI mass spectrum of the orange-colored substance exhibited a molecular ion peak at m/z 221 (MH^+). 1H NMR spectrum showed a singlet at $\delta=6.90$ ppm and one three-spin system. ^{13}C DEPT measurements indicated the presence of eleven carbons: four different C-H carbons and seven different quaternary carbons. This result was consistent with a high-resolution FAB mass spectrometric measurement of the $[M-H]$, which confirmed the exact elemental composition of the product to be $C_{11}H_7O_5$. IR spectrum showed the presence of carbonyl and hydroxyl groups. From all these results, the structure was unambiguously assigned

to be purpurogallin.

In order to confirm that the orange-colored metabolite of pyrogallol produced by human erythrocytes was purpurogallin and that purpurogallin might be produced by the reaction of human hemoglobin with pyrogallol, we studied the patterns of thin layer chromatography of the samples obtained by the reaction of pyrogallol with human erythrocytes or with crude oxyhemoglobin. Fig. 4 shows the thin layer chromatographic patterns of these samples, with authentic pyrogallol and purpurogallin. It was found that the product of pyrogallol with human erythrocytes or with human crude oxyhemoglobin was purpurogallin, since its chromatographic mobility was in good accordance with that of authentic purpurogallin.

Since we showed that purpurogallin was produced during the oxidation of oxyhemoglobin and reduction of methemoglobin by pyrogallol,

TABLE 2. Data of APCI-MS, NMR and IR spectra of the sample

APCI-MS: m/z 221 (MH^+)

HRMS calculated for $C_{11}H_7O_5$ 219.0287, found 219.0305 ($M - H$)

1H NMR (300 MHz, $DMSO-d_6$): δ 7.35 (d, 11.2 Hz, 1H), 7.08 (d, 9.3 Hz, 1H), 6.90 (s, 1H), 6.74 (dd, 11.2 and 9.3 Hz, 1H)

^{13}C NMR (76 MHz, $DMSO-d_6$): δ 182.3, 154.7, 151.8, 151.6, 134.8, 134.4, 133.1, 123.7, 116.6, 114.9, 110.3

IR(KBr): 3416, 3322, 1626, 1590, 1423, 1378, 1235, 1066, 1036, 1007, 845, 802 and 649 cm^{-1}

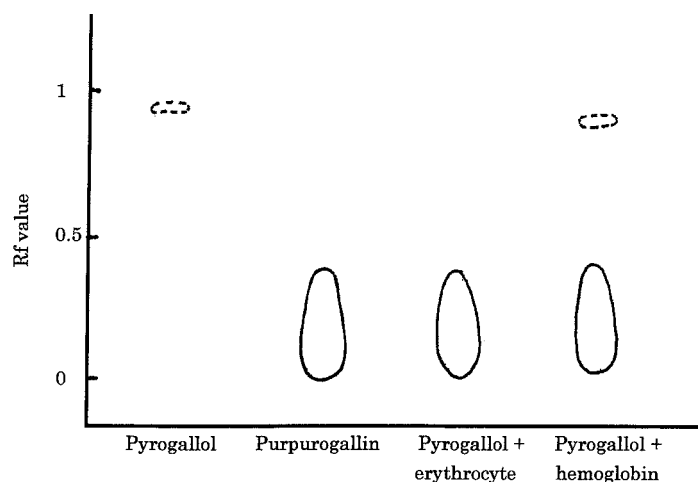


Fig. 4. Thin layer chromatography of the samples obtained after incubation of human erythrocytes or human crude oxyhemoglobin with pyrogallol.

TABLE 3. *The rate of oxido-reductive reactions of human hemoglobin with purpurogallin in comparison with that with pyrogallol*

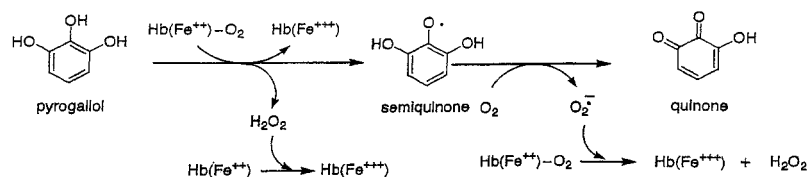
Oxidation rate of oxyhemoglobin by pyrogallol and purpurogallin ($\mu\text{M heme}/\text{min}$)		
	pyrogallol	purpurogallin
Control	0.85	0
+ catalase, superoxide dismutase	0.1	0
Reduction rate of methemoglobin by pyrogallol and purpurogallin ($\mu\text{M heme}/\text{min}$)		
	pyrogallol	purpurogallin
Control	0.92	4.04
+ catalase, superoxide dismutase	0.13	4.04

we examined whether purpurogallin may oxidize oxyhemoglobin and reduce methemoglobin. As shown in Table 3, purpurogallin did not oxidize oxyhemoglobin in the presence or absence of superoxide dismutase and catalase, however, it reduced methemoglobin greatly. The reduction rate of methemoglobin by purpurogallin ($4.04 \mu\text{M heme}/\text{minute}$) was about 31 times faster than that by pyrogallol in the presence of superoxide dismutase and catalase ($0.13 \mu\text{M heme}/\text{minute}$).

DISCUSSION

There are few descriptions on the detailed oxido-reductive reactions of hemoglobin with pyrogallol, though this compound has been used for staining leather, treatment of psoriasis and

measurement of superoxide dismutase activity (Gao et al. 1998). We found that purified human oxyhemoglobin was oxidized and methemoglobin was reduced by pyrogallol (Figs. 1 A and B, Table 1). Since these reactions were much suppressed in the presence of superoxide dismutase and catalase, it is conceivable that active oxygens such as superoxide and hydrogen peroxide were involved in these reactions. A plausible mechanism for the oxidation of oxyhemoglobin by pyrogallol may be proposed as the process shown in Scheme 1. Namely, oxyhemoglobin will be oxidized by pyrogallol to ferric form producing hydrogen peroxide (H_2O_2) as has been suggested for the mechanism of oxyhemoglobin oxidation by some reductants such as phenol and hydroquinone by Wallace and



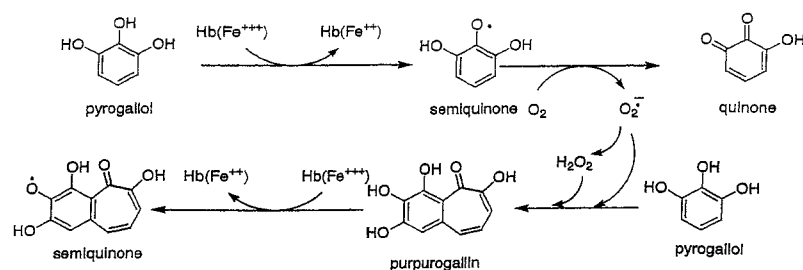
Scheme 1. Possible process of oxidation of human hemoglobin by pyrogallol

Caughey (1975). In this case, semiquinone form of pyrogallol, which is shown easily to react with oxygen to produce quinone form of pyrogallol and superoxide (O_2^-), will be produced. H_2O_2 and O_2^- , thus produced, oxidize ferrous hemoglobin to ferric hemoglobin (Eyer et al. 1975; Sutton et al. 1976). Therefore, the oxidation rate of oxyhemoglobin will be suppressed by scavenging these active oxygens in the presence of superoxide dismutase and catalase.

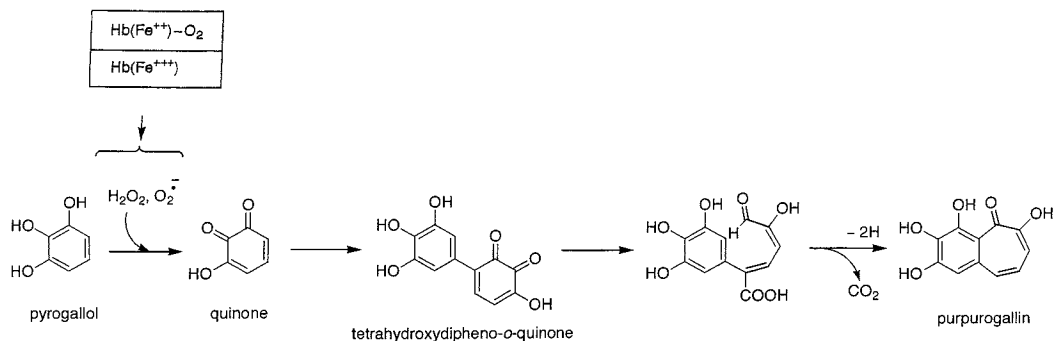
On the other hand, the reduction of methemoglobin by pyrogallol is complicated because the reaction rate was extensively suppressed by superoxide dismutase and catalase. However, we found that purpurogallin, which is produced during methemoglobin reduction by pyrogallol (Fig. 4), has strong activity of reducing methemoglobin, *i.e.*, purpurogallin reduced methemoglobin much more rapidly in the absence of catalase and superoxide dismutase than pyrogallol in the presence of catalase and superoxide dismutase (Table 3). Taking account of the fact that pyrogallol reacts with O_2^- and H_2O_2 , producing purpurogallin (Gao et al. 1998), we propose the process shown in Scheme 2 for the mechanism of methemoglobin reduction by pyrogallol. Firstly, pyrogallol, as an one electron reductant, will reduce methemoglobin directly producing semiquinone form of pyrogallol, which is further oxidized to the quinone form of pyrogallol accompanying the production of O_2^- . Superoxide (O_2^-) is easily converted to H_2O_2 by dismutation, especially in human erythrocytes, where superoxide dismutase is abundantly present. These active oxygens will induce the conversion of pyrogallol to purpurogallin as described in (Gao et al. 1998). Thus, the reduction rate of methemoglobin by pyrogallol will be suppressed in the presence of superoxide dismutase and catalase, due to extensive suppression of the production of purpurogallin with strong reducing capacity.

The effect of P_6 -inositol on the oxidation and reduction rates of hemoglobin with pyrogallol may be explained by the conformational changes in oxyhemoglobin and methemoglobin by this organic phosphate, as suggested by Perutz et al. (1974). Such acceleration of the reaction rates caused by P_6 -inositol has been indicated in the oxido-reductive reactions of hemoglobin with various oxidants and reductants (Tomoda et al. 1977b; Tomoda and Yoneyama 1979).

We found that pyrogallol was quickly metabolized to orange-colored compound in human erythrocytes coupled with the oxidation of



Scheme 2. Possible process of reduction of human methemoglobin by pyrogallol



Scheme 3. Possible process of pyrogallol metabolism to purpurogallin by human hemoglobin

intracellular hemoglobin. This compound was identified as purpurogallin (Table 2 and Fig. 4). We also found that purpurogallin was produced by the reactions of pyrogallol with human oxy- or methemoglobin (Fig. 4). The reaction mechanism for pyrogallol metabolism due to human hemoglobin will be explained by the promotion of oxidation of pyrogallol by active oxygens such as O_2^- and H_2O_2 which are produced in large quantities during the oxidation of oxyhemoglobin or the reduction of methemoglobin with pyrogallol (Figs. 1 A and B), because pyrogallol is demonstrated to be oxidized to purpurogallin by O_2^- (Gao et al. 1998) or by H_2O_2 at weak alkaline pH (Critchlow et al. 1967). Thus, it is possible that O_2^- and H_2O_2 produced by the reaction of oxyhemoglobin or methemoglobin with pyrogallol may oxidize pyrogallol to quinone form, which is postulated to be dimerized to purpurogallin via tetrahydroxydiphenyl-o-quinone accompanying the release of carbon dioxide, as Critchlow et al. (1967) suggested. From these results, the reaction of pyrogallol with human hemoglobin would be proposed as Scheme 3.

Though it is well known that hemoglobin acts as an oxygen carrier in human erythrocytes, the contribution of this hemoprotein to drug metabolism was not sufficiently recognized except for several reports (Mieyal and Blumer, 1976; Tomoda et al. 1977a; Ferraiolo et al. 1984). Since drugs absorbed in the human body may be primarily exposed to erythrocytes circulating in the blood, before reaching the liver, where many

drugs are detoxified by cytochrome P-450, our results suggest that hemoglobin in erythrocytes may play an important role in detoxifying some drugs, such as pyrogallol. We previously showed that *o*-aminophenol and its derivatives were metabolized to phenoxazines in human erythrocytes or by hemoglobin, and that these phenoxazines show less adverse effects *in vivo* using mice (Mori et al. 2000), which agrees with the results of Eckert and Eyer (1983) that *o*-aminophenol does not exhibit nephrotoxicity due to rapid formation of the phenoxazines in dogs. Metabolism of drugs by hemoglobin have been reported by some authors. Namely, Kühn et al. (1981) showed that hemoglobin catalyzes the peroxidation reaction of linoleic acid. Dealkylation of *N*-dimethylamine-*N*-oxide and decarboxylation of DOPA by hemoglobin were shown by Kiese et al. (1971) and Yamabe and Lovenberg (1972), respectively. Erythrocytic hemoglobin was reported to metabolize leukotriene A_4 to leukotriene B_4 (Fitzpatrick et al. 1984). The present results also characterize the role of human hemoglobin in drug metabolism. Especially, since purpurogallin has recently found as beneficial activities to protect cardiovascular cells (Wu et al. 1996) by scavenging hydroxyl radicals, the research of purpurogallin produced by the reactions with human hemoglobin or human erythrocytes may contribute to the elucidation of the mechanism of action of purpurogallin, and that of production of purpurogallin in the human body.

Acknowledgments

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