in vivo *20*: 845-848 (2006)

Kinetic Radical-scavenging Activity of Platonin, a Cyanine Photosensitizing Dye

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Abstract. *Platonin is known to possess antioxidant activity. However, the kinetics of the radical-scavenging activities of this compound remain unknown. The aim of this study was to investigate the radical-scavenging activities of platonin by the induction period method in the polymerization of methyl methacrylate (MMA), initiated by thermal decomposition of 2,2'-azobis (isobutyronitrile) (AIBN) (a carbon-centered radical, R•), and benzoyl peroxide (BPO) (an oxygen-centered radical, PhCOO•), under nearly anaerobic conditions. The number of moles of R• or PhCOO• radicals trapped by platonin calculated with respect to 1 mole of inhibitor moiety unit (stoichiometric factor, n) was determined, and this showed that the n of fully oxidized platonin was 4. The inhibition rate constant (k_{inh}) of platonin showed a wide range of $0.8x10^3$ $M^{-1}s^{-1}$ to $1.6x10^4$ $M^{-1}s^{-1}$. To clarify the interaction between platonin and thiols, 2-mercapto-1-methylimidazole (MMI) was used as a representative thiol, because glutathiones were unsuitable due to their limited solubility in MMA. MMI in the presence of platonin showed neither catalytic activities nor synergistic activities. Platonin possesses radical-scavenging activities and acts as an antioxidant. On the basis of our experimental results, the radical-scavenging mechanism is discussed.*

Platonin, a cyanine photosensitizing dye, has been previously reported to have antimicrobial activities (1-3) and pharmacological properties, such as promotion of burn-healing (4) and antihistaminic activity (5). Platonin has a beneficial effect in ameliorating endotoxemia in rat models, possibly through a reduction of mean arterial blood pressure and inhibition of NO and free radical formation (6). Platonin inhibits the production of pyrogenic cytokines from human peripheral blood mononuclear cells, resulting in antipyresis (7), and is a potent macrophage-activating agent and immunomodulator. The photodegradation products have been determined previously using gas chromatography-mass spectrometry, and the cytotoxicity of platonin and its photo-irradiated form against rat hepatocytes has been investigated (8). Platonin fades in the presence of light and oxygen, and loses its biological activities, generating reactive oxygen species, heptyl radicals and heptyl cations. The cytotoxicity of platonin is due to the radicals it produces, and not to its degradation products (8). On the other hand, platonin also possesses antioxidant activity. Photosensitizing compounds, such as platonins, scavenge lipid radicals derived from auto-oxidation of methyl linoleate (9). Also, platonin reduces the ESR signal intensity of superoxide anions, hydroxy radicals and methyl radical formation (7). Although platonin possesses antioxidant activity, the kinetics of the reaction of platonin with carbon-centered or oxygen-centered radicals under anaerobic conditions remain unknown. For maximum biological relevance, studies on platonin should be performed under anaerobic conditions, because biological systems have a low oxygen tension (10).

A quantitative model rationalizing the radical-scavenging activity of butyrated hydroxytoluene-related quinones (11), β-carotenes (12), polyamines (13), polyphenols (14), melatonin (15) and ebselen (16) in polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2,2'-azobis (isobutyronitrile) (AIBN) (a carbon-centered radical, R•) or benzoyl peroxide (BPO) (an oxygen-centered radical, PhCOO•) under nearly anaerobic conditions has been previously proposed. This model was able to explain the mechanism of the radical-scavenging activity of bioactive compounds (11-16). In the present study, this approach was extended to the investigation of platonin, and kinetic studies of the radical-scavenging activity of platonin are reported.

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Key Words: Platonin, antioxidants, stoichiometric factors, inhibition rate constants, benzoyl peroxide, azoinitiators.

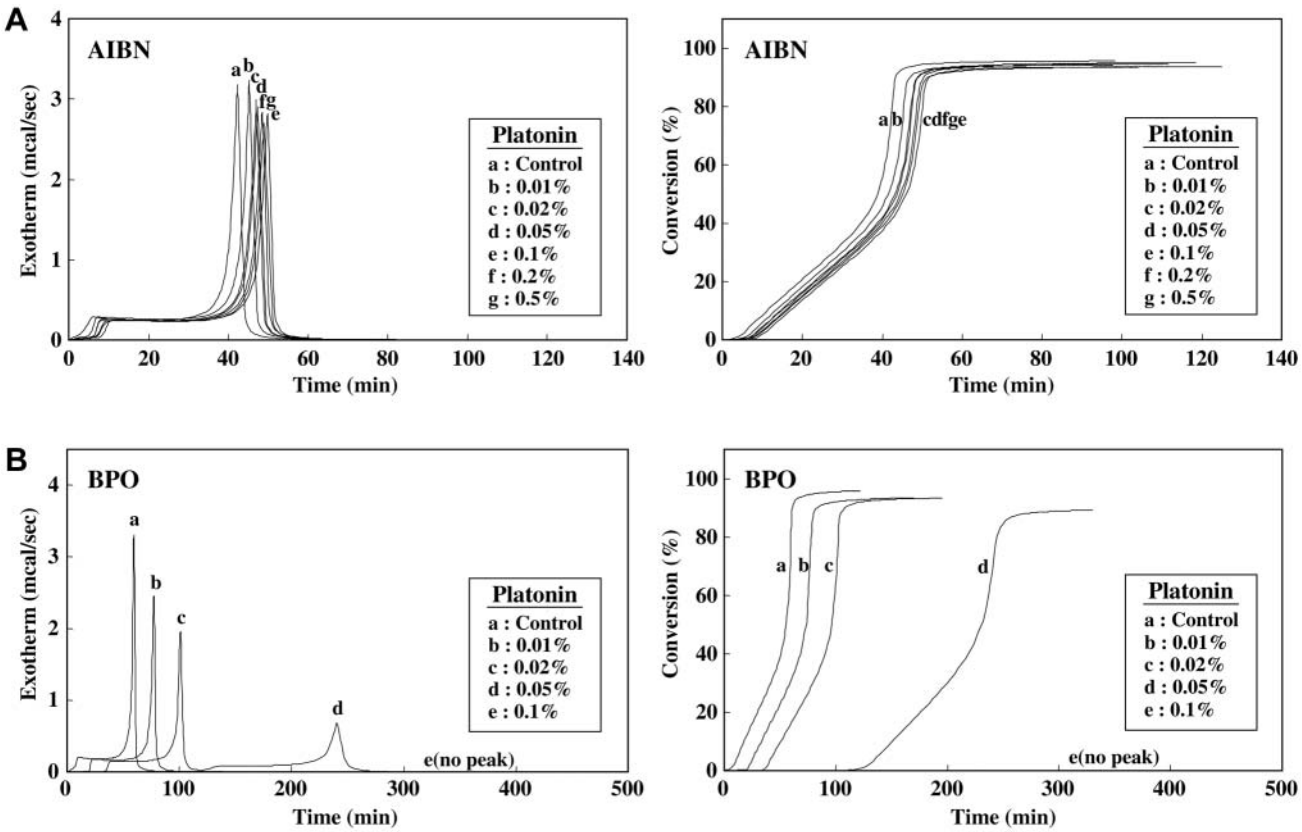

Figure 1. *Time-exotherm and time-conversion curves of platonin for the AIBN (A) and BPO (B) systems.*

Materials and Methods

Platonin was obtained from the Japanese Research Institute for Photosensitizing Dyes Co. Ltd., Okayama, Japan. MMI (2-mercapto-1-methylimidazole), AIBN and BPO were obtained from Tokyo Kasei Chem. Co. AIBN and BPO were recrystallized from methanol and methanol/chloroform (1:1 v/v), respectively.

Induction period (IP) and initial rate of polymerization. The IP and initial rate of polymerization in the presence (Rp_{inh}) or absence (Rp_{con}) of an antioxidant were determined by a previously reported method (11). In brief, the experimental resin consisted of MMA and AIBN (or BPO) with or without additives. AIBN (or BPO) was added at 1.0 mol%, and the additives were used at 0, 0.01, 0.02, 0.05, 0.1, 0.2 or 0.5 mol%. Approximately 10 μl of the experimental resin (MMA: 9.12-9.96 mg) was loaded into an aluminum sample container and sealed by applying pressure. The container was placed in a differential scanning calorimeter (DSC; model DSC 3100; MAC Science Co., Tokyo, Japan) kept at 70˚C, and the thermal changes induced by polymerization were recorded for the appropriate periods. The heat due to polymerization of MMA was 13.0 kcal/mole in this experiment. The conversion of all samples, as calculated from DSC thermograms, was 92-95%. Polymerization curves were derived from the DSC thermograms using the integrated heat evoked by the polymerization of MMA. The polymerization curves showed breaks when an inhibitor was consumed (Figure 1). These breaks were sharp and provided a reliable measure of the IP of the inhibitor. The presence of oxygen retards polymerization, because oxygen reacts with MMA radicals activated by the initiator and then subsequently produces a non-radical product. Thus, polymerization of the control was slightly inhibited, even though the reaction was carried out in a sealed DSC pan, because – as it had been sealed in air – the pan contained a small amount of oxygen. Tangents were drawn to the polymerization curves at an early stage in the run. The IP of the test compounds was determined from the length of time between the zero point on the abscissa and the point of intersection of tangents drawn to the early stage of polymerization. The IP was calculated from the difference between the induction period of specimens and that of controls. The initial rates of polymerization Rp_{con} and Rp_{inh} of platonin were calculated from the slope of the plots of the first linear line of the conversion rate of MMA polymerization (tangent drawn at the early polymerization stage).

Measurement of stoichiometric factor (n). The relative *n* value in Eq. **[1]** can be calculated from the induction period in the presence of an inhibitor:

$$n = R_i[IP]/[IH] \quad [1]$$

where [IP] is the induction period in the presence of an inhibitor [IH], and R_i is the rate of decomposition of the initiators. The R_i values of AIBN and BPO were 5.66×10^{-6} Ms^{-1} and 2.28×10^{-6} Ms^{-1}, respectively (11).

Table I. *Radical-scavenging activities of platonin with or without 2-mercapto-1-methylimidazole (MMI).*

Initiator	Additives	IP (min)	Rp_{inh}/ Rp_{con}	*n*	k_{inh}/ k_p
BPO	0.01 mol% Platonin	13.89	0.87	2.2	8.70
BPO	0.02 mol% Platonin	17.71	0.77	2.3	6.44
BPO	0.05 mol% Platonin	118.31	0.43	3.8	2.07
BPO	0.1 mol% Platonin	np			
BPO	0.01 mol% Platonin+				
	0.01 mol% MMI	16.1	0.75		
BPO	0.01 mol% MMI	1.97	0.77	0.3	63.09
AIBN	0.01 mol% Platonin	1.90	1.01	0.8	39.12
AIBN	0.02 mol% Platonin	2.95	0.97	0.5	26.86
AIBN	0.05 mol% Platonin	3.01	0.91	0.4	27.41
AIBN*	0.001 mol% Platonin	4.71	0.83	1.8	
AIBN	0.01 mol% Platonin+				
	0.01 mol% MMI	3.13	0.93		
AIBN	0.01 mol% MMI	0.50	1.01	0.2	149.21

IP, induction period; BPO or AIBN, 0.1 mol%; MMA, 9.4 mol/l; *n*, stoichiometric factor; np, no polymerization after curing for 999 min at 70 centigrade.

The Rp_{inh} and Rp_{con} are initial rate of polymerization with and without an inhibitor, respectively. The k_{inh} and k_p are the rate constant of inhibition and propagation, respectively. *The IP_{con} and Rp_{con} for 0.01 mol% AIBN were 15.29 min and 0.51 %/min, respectively. The IP_{con} (Rp_{con}) for BPO and AIBN were 6.71 min (0.95 %/min) and 3.48 min (1.33%/min), respectively. The IP subtracted control's IP from observed IP. The values are means for three independent experiments. The computational error was <7%. The procedures are described in the text.

Measurement of inhibition rate constant (k_{inh}). When R_i is constant, *i.e.,* when new chains are initiated at a constant rate, a steady-state treatment can be applied and the initial rate of polymerization of MMA is given by Eq. **[2]** (13-16):

$$Rp_{con} = \{k_p[MMA]\ R_i^{1/2}\}/(2k_t)^{1/2} \quad \textbf{[2]}$$

where K_p is the propagation rate constant.

The R_{pinh} rates are determined by Eq. **[3]**.

$$Rp_{inh}=\{k_p\ [MMA]\ R_i\}/\{n\ k_{inh}\ [IH]\} \quad \textbf{[3]}$$

in which R_{pinh} is the initial rate of inhibited polymerization, [MMA], *n*, [IH] and k_p are defined above, and k_{inh} is the rate constant for scavenging (inhibiting) of MMA radicals by an antioxidant. From Eq. **[2]** and Eq. **[3]**, k_{inh}/k_p can be calculated (Eq. **[4]**):

$$k_{inh}/k_p = [MMA]/\{[IP]x[Rp_{inh}]\} \quad \textbf{[4]}$$

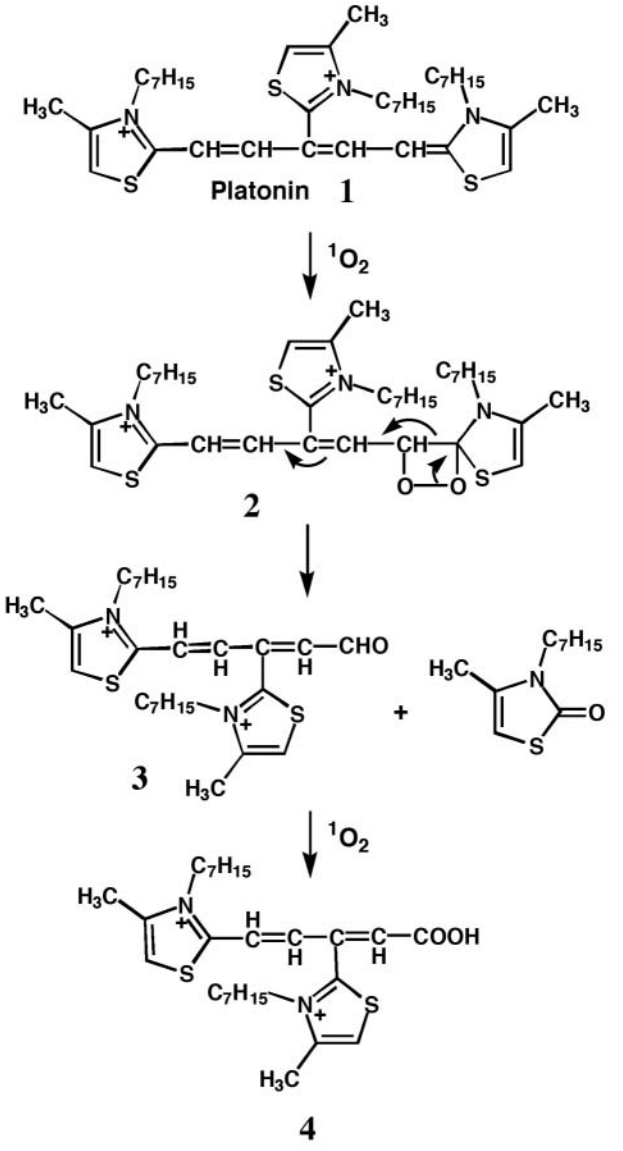

Figure 2. *Possible mechanism for oxidation of platonin by $^1O_2$. Compound **1**, platonin. Intermediates, compounds **2-4** were estimated on the basis of the results for fragmentation of photodegradation products of platonin (8).*

Results and Discussion

IP. Time-exotherm and -conversion curves for platonin for the AIBN (A) and BPO (B) system, respectively, are shown in Figure 1; the results are shown in Table I. Before sealing, the DSC sample pans contained a test material in air at room temperature, and the blue color of the platonin/MMA mixture turned dark red immediately after addition of BPO, whereas the corresponding AIBN mixture remained blue, like the platonin/MMA mixture. Platonin produces a dark red color when oxidized, and, thus, very slight but significant oxidation of platonin occurs in the presence of BPO under room lighting in air, although BPO undergoes decomposition at about 65˚C to produce PhCOO•. This may be due to the fact that radicals derived from BPO are oxygen-centered. Platonin changes to a red dark color in the presence of $^1O_2$ (8), and is sensitive to light but not to heat (17).

The *n* values of platonin calculated from Eq. **[1]** are summarized in Table I. The *n* values of platonin for the BPO system were greater than those for the AIBN system.

Relative *n* values of platonin were within the range 1-4. The *n* value for fully oxidized platonin was 4. The *n* values are dependent on the R_i of the initiators and the radical-scavenging capacity of the antioxidants.

Next, the interaction between platonin and MMI was studied and the results are shown in Table I. The IP of the platonin/MMI mixture was similar to that of the simple sum of platonin and MMI in both systems, indicating that MMI showed neither suppression of radical-scavenging activity nor promotion of synergistic activity of platonin. These results suggest that platonin may not be affected by glutathiones during oxidation in biological systems.

k_{inh}/k_p. The k_{inh}/k_p value of platonin was calculated from Eq. **[4]**, and the results are shown in Table I. The k_{inh}/k_p value of platonin for the BPO system declined as concentrations increased, whereas the k_{inh}/k_p for the AIBN system was constant and was similar to that of the controls. Platonin oxidized by BPO suppressed the production of MMA radicals, judging from the decrease in Rp_{inh}. At a 10:1 molar ratio of BPO/platonin, polymerization did not occur even after curing for 999 min. At a concentration of 0.01 mol%, the k_{inh}/k_p value of platonin for the AIBN system was about 7-fold greater than that for the BPO system, whereas the k_{inh}/k_p value of MMI for the corresponding systems was twice as large. This suggested that platonin was much more sensitive to oxygen-centered radicals than to alkyl radicals. At concentrations of 0.01-0.05 mol%, the k_{inh} of platonin for the AIBN and BPO systems was $(1.1\text{-}1.6)\times10^4$ $M^{-1}s^{-1}$ and $(0.8\text{-}3.5)\times10^3$ $M^{-1}s^{-1}$, respectively [note: k_p is 405 $M^{-1}s^{-1}$ (15)]. The k_{inh} of platonin lay within a wide range of 0.8×10^3 $M^{-1}s^{-1}$ – 1.6×10^4 $M^{-1}s^{-1}$. This value was similar to that of melatonin (15) and about 10-fold less than that of β-carotene (12).

Photodegradation products of platonin have been reported previously (8); on this basis, a possible mechanism for the radical-scavenging activities of platonin has been proposed, as shown in Figure 2. At the initial step, platonin (**1**) scavenges two free radicals and produces an intermediate, **2**, and subsequently compound **2** transforms to compound **3**. A further radical oxidation leads to compound **4** from compound **3**, due to radical oxidation of a CHO moiety to a COOH moiety. The *n* value for fully oxidized platonin becomes 4. The experimental result was in agreement with that estimated from the fragmentation of platonin degradation products (8).

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Received July 11, 2006
Accepted September 13, 2006