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Kinetic Evaluation of Polyamines as Radical Scavengers

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Abstract. *To clarify whether polyamines scavenge alkyl (carbon-centered) and peroxy (oxygen-centered) radicals, we analyzed their effects on the kinetics of polymerization of methyl methacrylate (MMA) induced by 2, 2'-azobisisobutyronitrile (AIBN, a R· radical) and benzoyl peroxide (BPO, a PhCOO· radical) under nearly anaerobic conditions. Stoichiometric factors (n; number of free radicals trapped by one mole of antioxidant moiety) were determined by the induction period method. The n value for polyamines (putrescine, spermidine and spermine) was 0.1-0.7, whereas that for conventional synthetic antioxidants, BHA and BHT, was about 2. These n values were not different between the AIBN and BPO systems. The n value for polyamines declined in the order spermine>spermidine >putrescine. The K_{inh}/K_p value for polyamines (20-115) was greater than that (4-7) for BHT or BHA. Radical-scavenging activity largely depends on the stoichiometric factor of antioxidants rather than their effects on initial rate of polymerization, a rate of propagation. Polyamines may scavenge alkyl or peroxy radicals derived from polyunsaturated fatty acids in biological systems.*

The naturally occurring polyamines, spermine, spermidine and the diamine putrescine are widespread in nature. They have been implicated in growth and differentiation processes. Polyamines accumulate in cancerous tissues and their concentration is elevated in body fluids of cancer patients (1). Polyamines have been previously reported to have antioxidant, anti-inflammatory and other activities (2, 3). However, it poorly understood whether polyamines act as antioxidants in cells. Polyamines are useful for the protection of easily oxidizable compounds, especially polyunsaturated fatty acids (3), and may be applicable for the protection of marine oils such as cod liver oil, capelin oil and their concentrates, and various foodstuffs, against oxidation (3). On the other hand, polyamines are inefficient scavengers of radicals such as HO·, t-BuO· and O_2^-, and ROO· derived from AAPH (2, 2'-azobis (2-amidinopropane) dihydrochloride) compared with potent antioxidants such as vitamin C and vitamin E (4, 5). Polyamines may inhibit oxidation by biological catalysts containing metal ions as a result of complex formation between the polyamines and metal ions such as Fe^{2+} and Cu^{2+} (5-7). In contrast, polyamines are good scavengers of hydroxyl radicals (8). These discrepancy may be attributed to experimental conditions, particularly oxygen tensions. The oxygen tension under a 15 torr oxygen atmosphere is similar to that in many tissues, suggesting that oxygen is sparse in cells (9, 10). Previous quantitative *in vitro* studies of the radical-scavenging activity of some amines were carried out under aerobic conditions, although oxidation of amines is much more complicated than that of phenols (11, 12). Thus, we believe that the efficiency of antioxidants may be different in biological conditions under low oxygen tensions. We have previously used differential scanning calorimetry (DSC) and induction methods to investigate the radical-scavenging efficiency of estrogen-like bisphenol-A compounds (13), polyphenols (14, 15), polyenes (16) and butylated hydroxytoluene-related quinones (17) under nearly anaerobic conditions, and this method has proved to be reliable in evaluating the activity of these antioxidants.

In the present study, we investigated the radical-scavenging activity of polyamines (putrescine, spermidine and spermine) and conventional synthetic antioxidants such as 2, 6-di-*tert*-butyl-4-methylphenol (BHT) and 2-*tert*-butyl-4-methoxyphenol (BHA), by determining the induction period for polymerization of methyl methacrylate (MMA) initiated by thermal decomposition of 2, 2'-azobisisobutyronitrile (AIBN) or benzoyl peroxide (BPO).

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Key Words: Polyamines, stoichiometric factors (n), inhibition rate constants.

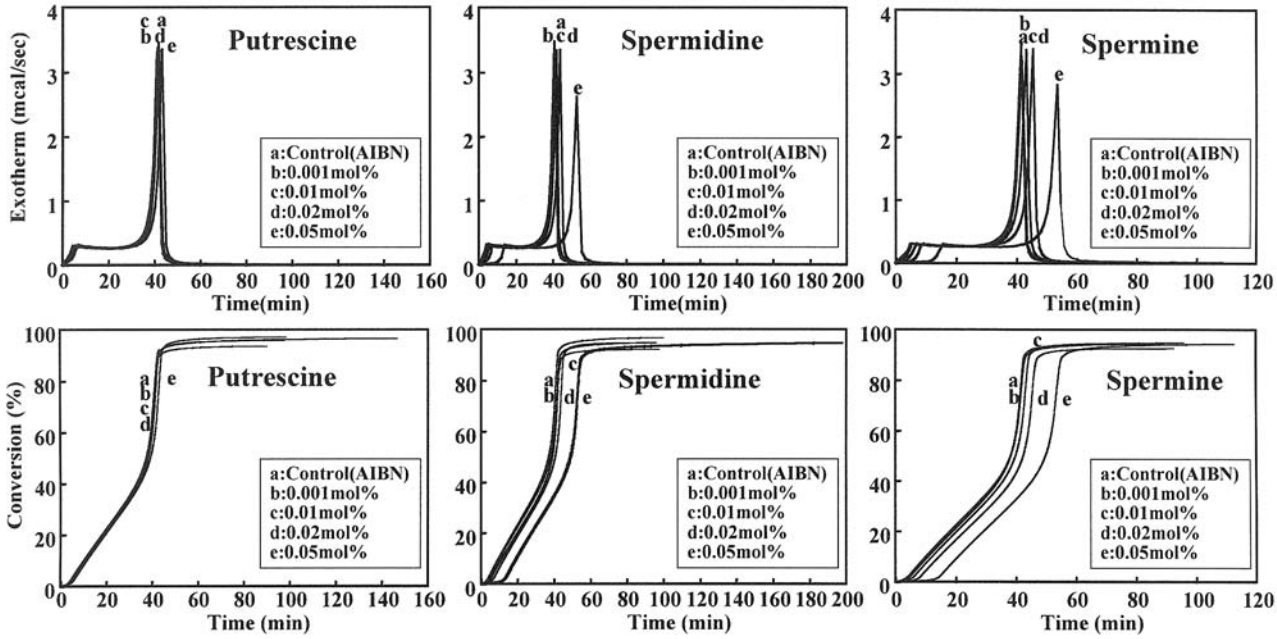

Figure 1. *Exothermic (top) and time-conversion (bottom) for the polymerization of MMA/AIBN in the presence of polyamine additives.*

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies. Putrescine, spermidine, spermine (Sigma Chemical Ind., St. Louis, MO, USA); BHT, BHA and MMA (Tokyo Kasei Chem. Co.). MMA was purified by distillation. AIBN and BPO (Tokyo Kasei Chem. Co.) were recrystallized from methanol and methanol/chloroform (1:1 v/v), respectively.

General experimental procedures. The induction period (IP) and initial rate of polymerization in the presence (Rp_{inh}) or absence (Rp_{con}) of an antioxidant were determined by the method previously reported (13-17). In brief, the experimental resin consisted of MMA and AIBN (or BPO) with or without additives. AIBN (or BPO) were added at 1.0 mol%, and the additives were used at 0.001, 0.01, 0.02 and 0.05 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 (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 92-96%. Polymerization curves were derived from DSC thermograms using the integrated heat evoked by the polymerization of MMA. Typical time-exotherm and time-conversion curves for polyamines for AIBN are shown in Figure 1. Polymerization curves break when an inhibitor is consumed. These breaks are sharp and provide 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 the pan contained a small amount of oxygen since it had been sealed in air. Tangents were drawn to polymerization curves at an early stage in the run. The IP of 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 in the absence (Rp_{con}) and presence (Rp_{inh}) of natural and synthetic antioxidants 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).

Rate of initiation (R_i) due to the thermal decomposition of AIBN or BPO was determined using Eq. 1.

$$R_i = n\,[IH]_0/[IP] \quad (1)$$

where $[IH]_0$ is the concentration of the inhibitor at time zero and [IP] is the induction period. 2, 6-Di-*t*-butyl-4-methoxyphenol (DTBM) was used to determine R_i, since its stoichiometric factor, *n*, is known to be 2.00(12) . In the case of [MMA] = 9.4 M and [AIBN or BPO] = 0.1 M at 70˚C, the induction period method using DTBM gave the rate of initiation, R_i, at 70˚C. The R_i values of AIBN and BPO were 5.66 x 10^{-6} Ms^{-1} and 2.28 x 10^{-6} Ms^{-1}, respectively(13-17). Stoichiometric factors (*n*) can be calculated from Eq. 2.

$$n = R_i[IP]/[IH] \quad (2)$$

where [IP] is the induction period in the presence of an inhibitor. The number of moles of peroxy radicals trapped by the antioxidant is calculated with respect to 1 mole of inhibitor moiety unit.

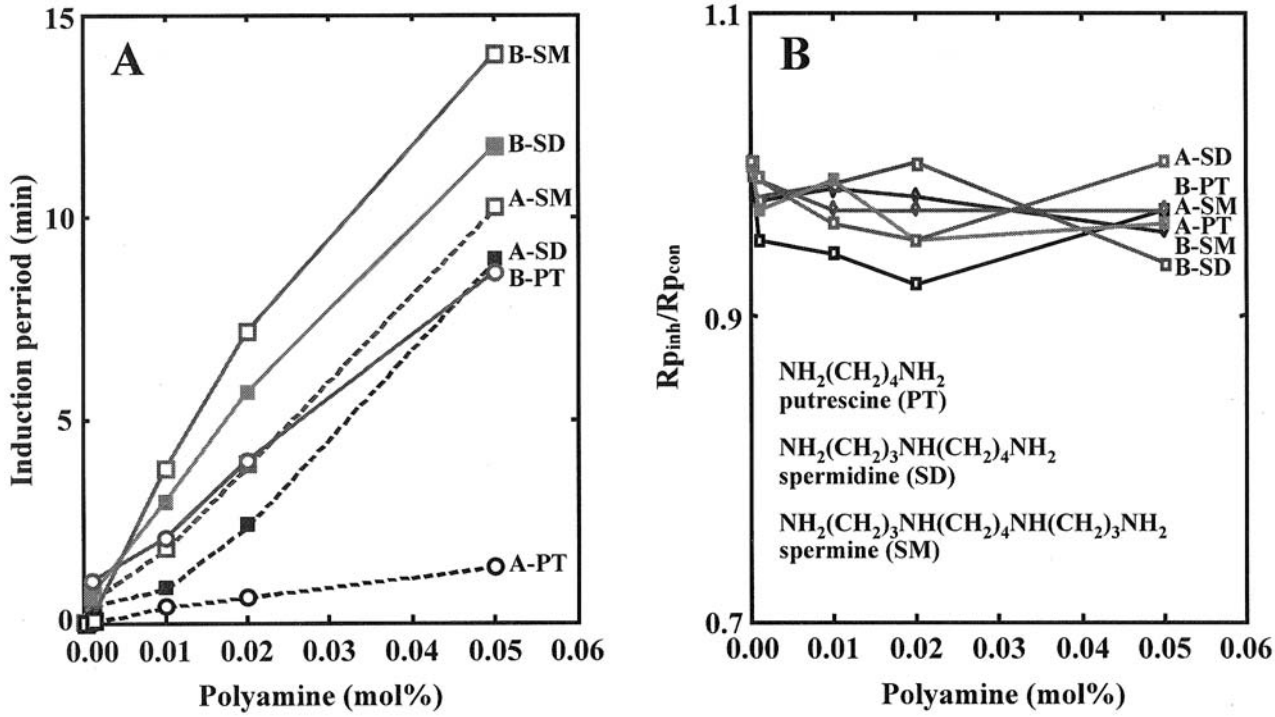

Figure 2. *Plots of induction period (A) and the Rp_{inh} /Rp_{com} (B) vs concentrations of polyamines in the polymerization of the MMA/AIBN and MMA/BPO systems. The inset is the chemical structure for spermine (SM), spermidine (SD) and putrescin (PT). A- and B-SM, SD and PT are polyamines in the AIBN and the BPO systems, respectively. The measurement is described in the text. Rp_{inh} and Rp_{con} are the initial rate of polymerization with polyamines and that without polyamines (control), respectively.*

When R_i is constant, *i.e.* when new chains are started at a constant rate, a steady-state treatment can be applied and the initial rate of polymerization of MMA is given by Eq. 3 (12, 17).

$$R_p = \{k_p [MMA] R_i^{1/2}\}/(2k_t)^{1/2} \quad 3$$

where MMA represents methyl methacrylate and k_p and k_t are the rate constants for chain propagation and termination, respectively.

The $k_p/(2k_t)^{1/2}$ rate of polymerization of MMA (9.4 M) by AIBN (1 mol%) and BPO (1 mol%) at 70°C was 9.86 x 10^{-2} $M^{-1/2}$ $s^{-1/2}$ (13, 17).

The Rp_{inh} rates are determined by Eq. 4.

$$Rp_{inh} = \{k_p [MMA] R_i\}/\{n\ k_{inh} [IH]\} \quad 4$$

in which Rp_{inh} 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. Eq. 2 and Eq. 4 can be rearranged to calculate k_{inh}/k_p.

$$k_{inh}/k_p = [MMA]/\{[IP] \times [Rp_{inh}]\} \quad 5$$

Results and Discussion

Stoichiometric factors (n). Typical time-exothermic (top) and time-conversion (bottom) curves for polyamines (putrescine, spermidine and spermine) for the AIBN systems are shown in Figure 1. The plots of IP (A) and the Rp_{inh}/Rp_{com} (B) *vs* concentrations for all antioxidants in both the AIBN-MMA and BPO-MMA systems are shown in Figure 2. The IP for both systems increased linearly in a dose-dependent manner. In the AIBN system, the radical-scavenging activity (IP) declined in the order spermine > spermidine > putrescine. In the BPO system, the scavenging activity of polyamines declined in a similar order to that in the AIBN system. The Rp_{inh} /Rp_{com} was linear, although the concentration increased. The radical-scavenging activity of all antioxidants is summarized in Table I. In both the AIBN and BPO systems, the *n* value (approximately 2) for BHT and BHA was greater than that (n= 0.1-0.7) for polyamines.

The inhibitory effect of polyamines on lipid peroxidation has been reported to be enhanced by increasing the number of N atoms, with activity increasing in the order putrescine < spermidine < spermine (3). The findings in the present study are in good agreement with that described above.

k_{inh}/k_p. Next, we examined the k_{inh}/k_p. The results are also shown in Table I. In the AIBN system, the k_{inh}/k_p value declined in the order of putrescine >spermidine>spermine >BHA> BHT. In the BPO system, the value declined in the order of putrescine >spermine> spermidine> BHA> BHT. The k_{inh}/k_p for polyamines (values, 20-115) was greater than that (values, 4-7) for the synthetic phenolic

Table I. *Induction perios (IP), stoichiometric factors (n), Rp_{inh}/Rp_{con}, and k_p/k_{inh} for polyamines, 2-t-butyl-4-methoxyphenol (BHA) and 2, 6-dit-butyl-4-methylphenol (BHT) in the AIBN- and BPO-MMA systems under nearly anaerobic conditions.*

Compounds	AIBN				BPO			
	IP (s)	n	Rp_{inh}/Rp_{con}	$k_{inh}/k_p \times 10^{-1}$	IP (s)	n	Rp_{inh}/Rp_{con}	$k_{inh}/k_p \times 10^{-1}$
Putrescine	39	0.11	0.97	11.46	233	0.31	0.92	3.24
Spermidine	146	0.41	0.95	3.24	341	0.46	1.00	2.08
Spermine	238	0.67	0.95	1.95	431	0.58	0.98	2.23
BHA	760	2.14	0.81	0.71	1610	1.83	0.79	0.54
BHT	620	1.78	1.0	0.72	1607	1.83	0.99	0.43

MMA, 9.4 mole/l; AIBN (or BPO), 0.1 mole/l;compound, 2 mmole/1; at 70˚C. The initial rate of polymerization with and without an inhibitor is Rp_{inh} and Rp_{con}, respectively. The Rp_{cont} for AIBN and BPO is 2.173×10^{-3} mole/l x s and 1.356×10^{-3} mole/l x s, respectively. The rate constant of inhibition and propagation is k_{inh} and k_p, respectively. The values were the mean of three independent experiments. Standard error <15%.

antioxidants, BHA and BHT. In particular, putrescine with a value of 115 in the AIBN system was the greatest among the test compounds. The k_{inh} for the polyamines and conventional synthetic antioxidants, BHA and BHT, was calculated (Note that the k_p value for MMA of 367 $M^{-1}s^{-1}$ at 60˚C was used for that at 70˚C, because the k_p of MMA at 70˚C is unknown but was assumed to be close to the value at 60˚C (18). It has been previously reported that the k_{inh} of putrescine (or spermidine) and spermine for *t*-BuO· radical is $<1.5 \times 10^5$ $M^{-1}s^{-1}$ and 4.2×10^6 $M^{-1}s^{-1}$, respectively (value is detection limit of system) (4). In the present study, the k_{inh} for polyamines was 7.2×10^4 $M^{-1}s^{-1}$ – 4.2×10^5 $M^{-1}s^{-1}$, whereas that for BHA and BHT was 1.6-2.6 x 10^4 $M^{-1}s^{-1}$. The k_{inh} for BHT is about 1 x 10^4 $M^{-1}s^{-1}$ (11, 12, 19). The present finding was in good agreement with that previously reported, although the methodologies were considerably different. This is probably attributable to the chemical structure of BHT with bulky-*tert*-butyl-substitutes at two *ortho*-positions, since BHT is less affected by molecular oxygen, due to the steric hinderance of substitutes (11).

It has previously reported that polyamines are poor scavengers of peroxy radicals, derived from the thermal decomposition of azo-initiator, suggesting low levels of chain-breaking activity (6). However, the present findings indicated that the k_{inh} value for polyamines was greater than that for conventional synthetic antioxidants, although the *n* for the former was considerably less than that for the latter. Several studies are available in which each of these two methods (the *n* and k_{inh}) was applied to elucidate the mechanism of a particular reaction, but there is no comparative study with respect to the scope and limitation of these two methods (19). In the present study, we examined the radical-scavenging activity using these two methods under nearly anaerobic conditions. Our results confirm that polyamines, particularly spermine, are efficient scavengers of both alkyl (R·) and benzoyl peroxyl radicals (PhCOO·) and also that putrescine shows a greater k_{inh} value. Intracellular concentration of putrescine were previously reported to be possibly a useful marker for the apoptosis induction or the sensitivity of the cells to apoptosis inducers because intercellular putrescines reduced under certain circumstances induced by reactive oxygen species (20). Thus, harmful peroxy radicals (ROO·) derived from polyunsaturated phospholipids in biological systems might be scavenged efficiently by polyamines.

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