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Oxidation of Tea Extracts and Tea Catechins by Molecular Oxygen

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Tea polyphenols (PP) are known as potent antioxidants. At the same time, PP have been repeatedly reported to oxidize by molecular oxygen with the formation of active forms of oxygen. In this work, the Clark electrode technique was applied to study the kinetics of the autoxidation of tea extracts and individual tea PP as well as model PP, catechol, gallic acid, and pyrogallol. Aqueous extracts of both green and black teas were found to undergo extensive autoxidation under physiological conditions. The addition of superoxide dismutase (SOD) and milk resulted in a significant decrease in the rate of oxidation. Studied individually, PP were found to autoxidize at a rate, which increased with pH, proportional to PP concentration and nearly proportional to oxygen concentration. The collected data were used for the extrapolation/interpolation of the starting rates of oxidation to the standard conditions (at pH 7.40, 100 μM PP, 200 μM O_2). PP oxidizability is basically determined by that of the key PP fragment (pyrogallol > gallate > catechol). Meta-OH groups do not contribute to the oxidation even at pH 13.0. Similar to tea brew, the oxidation of individual PP was inhibited by milk and SOD addition, with catechol being the only exception (the oxidation of catechol was accelerated when SOD was added). Comparison of the autoxidation of PP (*o*-hydroquinones) with that of *p*-hydroquinones (Roginsky, V.; Barsukova, T. K. *J. Chem. Soc., Perkin Trans. 2 2000*, 1575−1582) displays the dramatic difference both in the oxidizability and in the kinetic regularities. The difference in the kinetics has been suggested to be due to the difference in the initiation of the chain process. Whereas for *p*-hydroquinones the oxidation is initiated by the reaction between hydroquinone and a corresponding quinone, the oxidation of *o*-hydroquinones is likely started by direct interaction between substrate and molecular oxygen. As the second process is much slower, this may explain the relatively low oxidizability of PP as compared to *p*-hydroquinones.

KEYWORDS: Tea; polyphenols; catechins; oxidation; Clark electrode; chemical kinetics

INTRODUCTION

Tea extracts and individual tea polyphenols (PP) display both antioxidant and pro-oxidant effects. On the one hand, tea has been known for a long time as a beverage preventing many diseases including cardiovascular diseases (*1*) and cancer (*2*). The latter is commonly attributed to the pronounced antioxidant activity of tea PP (*3*−*5*). On the other hand, tea PP have been repeatedly reported as cytotoxic and mutagenic agents (*6*, *7*). This may be associated with pro-oxidant activity of tea PP caused by their tendency to autoxidation accompanied by the formation of active forms of oxygen. Although the autoxidation of tea PP has received sufficient attention in the literature, the quantitative information on the autoxidation of tea extracts and individual tea PP is rather limited. The main attention has been given to green tea and its major substituents, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (**Figure 1**). In particular, it was reported that tea brewing under severe conditions (15 min at 90 °C under agitation) was accompanied by the intensive generation of H_2O_2; subsequent incubation of tea extracts during several hours at room temperature resulted in further, but much slower, accumulation of H_2O_2 (*8*). The incubation of green tea brew under aerobic conditions during 1 h at pH 7.5 resulted in the dramatic decline in the level of some individual PP, first of all of EGCG (*9*). Works (*10*−*12*) give semiquantitative information on the kinetics of autoxidation of individual green tea catechins. The rate of autoxidation of EC, EGC, ECG, and EGCG was found to increase with pH and to be inhibited by superoxide dismutase (SOD) and catalyzed by Cu^{2+}.

In this work, the kinetic regularities of the autoxidation of tea brews and the main phenolic constituents of green tea were studied in detail by using the Clark electrode technique. Along with tea catechins, model PP, catechol, pyrogallol (PG), and

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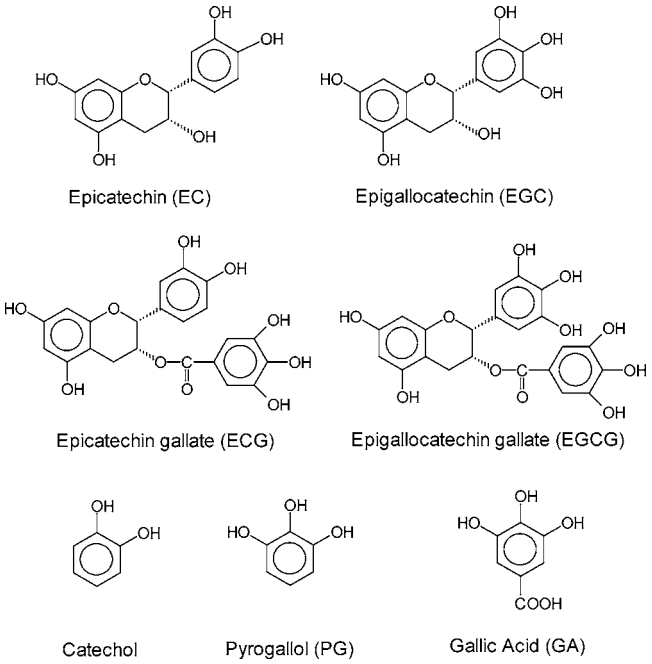

Figure 1. Green tea catechins and model polyphenols.

gallic acid (GA), which may be considered as key fragments of tea catechins, were also studied. The determination of the oxidizability of various tea catechins under the same conditions was among the main tasks of this work. For this purpose, the dependence of the rates of oxidation of PP on pH and reagent concentrations were studied, and the collected data were extrapolated/interpolated to the standard conditions: pH 7.40, 200 μM O_2, 100 μM PP, and 37 °C. To better understand the mechanism of catechin oxidation as well as the behavior of tea brew under interesting practical conditions, the effects of SOD and milk addition were also studied.

MATERIALS AND METHODS

Chemicals and Teas. Catechol, epicatechin, gallic acid, and epigallocatechin gallate were purchased from Aldrich. Catechin was from Fluka, myricetin and pyrogallol were from Sigma, and epigallocatechin and epicatechin gallate were obtained from Carl Roth. NaH_2PO_4 and Na_2HPO_4 used to prepare buffer solutions were purchased from Merck. All other chemicals were of the highest grade available. Aqueous solutions were prepared with doubly distilled water. The buffer solutions with pH within the range of 6.5−9.0 were prepared by mixing solutions of NaH_2PO_4 and Na_2HPO_4 without the addition of any acid or base. Solutions of the individual phosphates were prepared with doubly distilled water and were purged from traces of transition metals by Chelex-100 resin (Bio-Rad) using a batch method. Aqueous solution at pH 13.0 was prepared by dissolving KOH in distilled water. Stock solutions of individual PP were prepared, depending on solubility, with distilled water acidified with hydrochloric acid or with acidified aqueous DMSO. The following commercially available teas, both loose and in bags, were taken for the study: Messmer (green, bag); Ever Spring (green, bag); Chinese Gunpowder (green, loose); Shere (black, loose); Irish Breakfast (black, loose); Lipton (black, bag). Tea extracts (brew) were prepared by hanging a tea bag or placing 2.0 g of loose tea (weighed to ±0.0005 g) for 3 min at 100 °C in 100 mL of water, which was agitated with a magnetic stirrer. A 100 μL aliquot of brew was immediately added to testing solution (3.6 mL of 50 mM buffer, pH 7.40). In more detail, the protocol of tea brewing has been reported elsewhere (*13*).

Kinetic Measurements. The kinetics of oxygen consumption accompanying the oxidation of tea brew or PP were studied with a computerized 5300 Biological Oxygen Monitor (Yellow Springs Instruments Co., Yellow Springs, OH). When required, the system was bubbled with oxygen or argon during a few minutes prior to a run. The rate of oxidation was measured as a slope of $[O_2]$ trace. In more detail, the protocol of the determination of the kinetics of oxygen consumption has been described elsewhere (*13*−*15*).

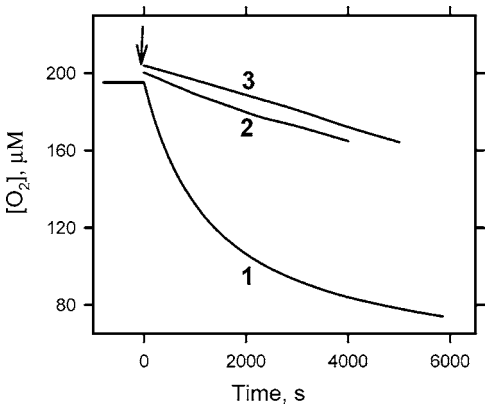

Figure 2. Kinetics of oxygen consumption accompanying the oxidation of brew of green tea Messmer in phosphate buffer, pH 7.40: trace 1, no addition; trace 2, + 100 units/mL SOD; trace 3, + 0.05 g of milk powder/mL of buffer. One hundred microliters of brew is added to 3.6 mL of buffer.

RESULTS

Oxidation of Tea Extracts. All of the studied tea extracts prepared both from green teas and from black teas showed pronounced oxygen consumption as exemplified in **Figure 2**. The rate of oxygen consumption (R_{OX}) decreased progressively with time. In the course of the run presented in **Figure 2**, plot 1, R_{OX} dropped from the starting value of 99 nM/s to 4.7 nM/s after 1.5 h of incubation. This reduction was basically due to the depletion of oxidizable phenolics but also the decrease of R_{OX} when oxygen concentration decreases (see below). When oxygen concentration was restored, R_{OX} increased from 4.7 to 15.3 nM/s (not shown). The starting values of R_{OX} for various samples of tea varied within rather narrow limits of 40−100 nM/s. There was no significant difference in the oxidizability between green and black teas. It should be noted that in the experiments presented in **Figure 2** we dealt with the oxidation of rather diluted tea extracts (~3% of original tea brew). This means that the starting value of R_{OX} for the real tea brews at pH 7.4 and 37 °C is expected to be 1−3 μM/s. The addition of SOD and milk brought about the significant decrease in R_{OX} (**Figure 2**, traces 2 and 3). For instance, the starting rate of oxidation of green tea Messmer decreased by factors of 10 and 8 in the presence of 0.05 g/mL milk powder and 100 units/mL SOD, respectively. These figures are typical of all other green and black teas.

Oxidation of Individual PP. The shape of O_2 traces was found to be similar for all of the studied PP [with one exception, catechol, under some conditions (see below)]. R_{OX} decreased progressively with time (**Figure 3**). In all cases, the starting value of R_{OX} was proportional to PP concentration as exemplified by **Figure 4**. The most credible reason for the decrease in R_{OX} with time is the progressive decrease in PP concentration. However, this may be, at least partly, due to the decrease in oxygen concentration during a run. To solve this problem, the starting value of R_{OX} was determined at various starting concentrations of O_2 with other conditions (pH, PP concentration) being the same. The results of these measurements for some PP are shown in **Figure 5**. It can be seen that the dependence of R_{OX} on $[O_2]$ is described in double-logarithmic axes by the straight line. This means that the above dependence

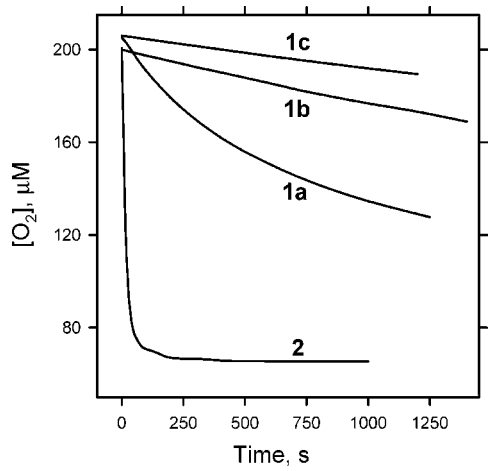

Figure 3. Kinetics of oxygen consumption accompanying the oxidation of EGC: traces 1a–c, 100 μM EGC, pH 8.00 (1a, no addition; 1b, + 40 units/mL SOD; 1c, + 0.05 g of milk powder/mL.); trace 2, 50 μM EGC, pH 13.0.

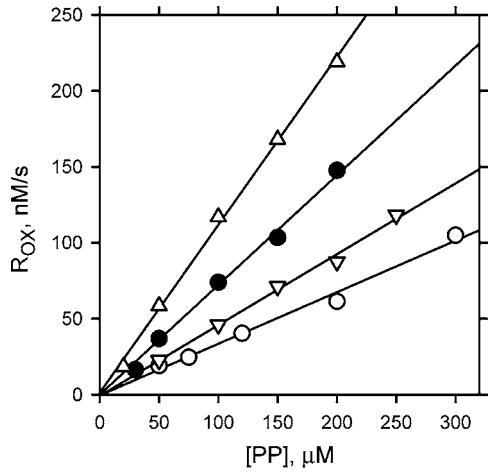

Figure 4. Concentration plots of the starting rates of the oxidation of individual PP: (●) PG, pH 7.40; (○) GA, pH 8.00; (△) EGC, pH 8.00; (▽) EGCG, pH 7.40.

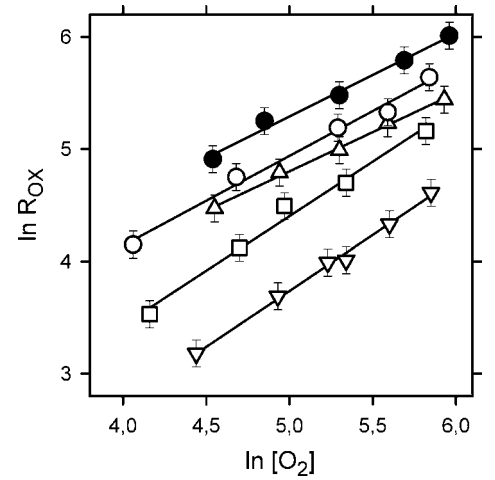

Figure 5. Plots of R_{OX} against oxygen concentration in the double-logarithmic axes: (●) 100 μM PG, pH 7.90; (○) 100 μM GA, pH 9.00; (□) 100 μM myricetin, pH 7.40; (△) 100 μM EGC, pH 8.00; (▽) 150 μM EGCG, pH 7.40.

may be given by the function $R_{OX} \sim [O_2]^{\beta}$. The parameter β was calculated from the slope of the plot of ln R_{OX} versus ln [O_2] (**Figure 5**). The values of β are listed in **Table 1**. Typically, β is close to 1; that is, R_{OX} is nearly proportional to oxygen concentration.

Table 1. Parameters of the Oxidation of Tea Catechins and Individual Model PP

polyphenol	effect of pH[a]	effect of $[O_2]$[b]	$(R_{OX})_{ST}$, nM/s[c]	effect of SOD[d]	n[e]
catechol	0.88	0.93 (9.0)	0.53 ± 0.09	>13	2.3 (13)
gallic acid	0.83	0.81 (9.0)	12.2 ± 2.1	0.32	2.8 (13)
pyrogallol	0.83	0.79 (9.0)	74 ± 8	0.14	2.1 (8.0–13)
epicatechin	nd[f]	nd	0.08 ± 0.02	0.36	2.5 (13)
catechin	nd	nd	0.21 ± 0.04	0.38	2.4 (13)
EGC	0.62	0.68	53 ± 5	0.22	1.4 (7.40); 2.9 (13)
ECG	0.86	0.85	9.2 ± 1.4	0.29	5.4 (13)
EGCG	0.85	1.00 (7.40)	42 ± 4	0.14	≥2.6 (9.0); 6.0 (13)
myricetin	0.96	0.96	117 ± 6	0.26	1.5 (7.40); 4.2 (13)

[a] Parameter α in the expression d(lg R_{OX})/d(pH) = α. [b] Parameter β in the expression $R_{OX} \sim [O_2]^{\beta}$ at pH indicated in parentheses. [c] Rate of PP oxidation under standard conditions (pH 7.40, 100 μM PP, 200 μM O_2). [d] Ultimate value of $(R_{OX})_{SOD}/(R_{OX})_0$, where $(R_{OX})_0$ and $(R_{OX})_{SOD}$ are the rate of PP oxidation without SOD and in the presence of SOD, respectively (see text for more detail). [e] Stoichiometric factors of oxygen consumption at pH indicated in parentheses. [f] Not determined.

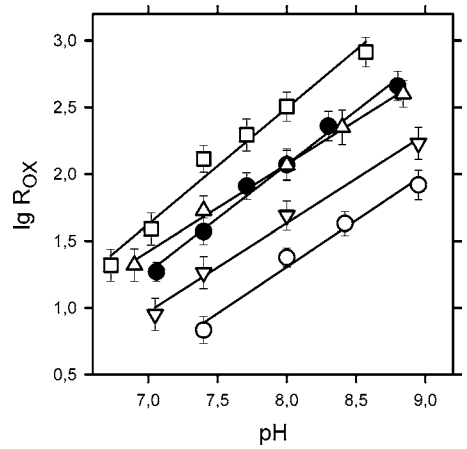

Figure 6. Effect of pH on starting rate of oxidation of various PP at $[O_2]$ = 200 μM: (●) 50 μM PG; (○) 50 μM GA; (□) 100 μM myricetin; (△) 100 μM EGC; (▽) 50 μM EGCG.

For all of the studied PP, R_{OX} was found to increase with pH (**Figure 6**). The dependence of R_{OX} on pH may be characterized by the parameter α in the equation d(lg R_{OX})/d(pH) = α. As seen from **Figure 6**, lg R_{OX} increased directly with pH. The values of α calculated from the slope of the plots of lg R_{OX} versus pH are listed in **Table 1**. They varied typically within rather narrow limits of 0.62−0.96. As for catechin and epicatechin, their oxidation even at pH <9 occurred too slowly and the value of α could not be determined.

The oxidation of studied tea PP as well as model PP occurred at a very high rate at pH 13.0 and was typically almost accomplished in a few minutes (**Figure 3**, trace 2). At the same time, PP containing two meta-OH groups in the C-ring, but only one (naringenin) or no OH group in the B-ring (chrysin), did not show any detectable oxygen consumption even at pH 13 (not shown). This is in line with work (*16*) reporting that 1,3,5-trihydroxybenzene, vanillic acid, and syringic acid did not oxidize even at pH 14. The starting value of R_{OX} at pH 13 typically correlates with the rate of oxidation determined at pH <9.0. Whereas the starting value of R_{OX} for 50 μM catechin was relatively low, 210 nM/s (not shown), the oxidation of 50 μM EGC occurred too quickly to measure the starting rate; this can be can be estimated only (>4000 nM/s, **Figure 3**, trace 2).

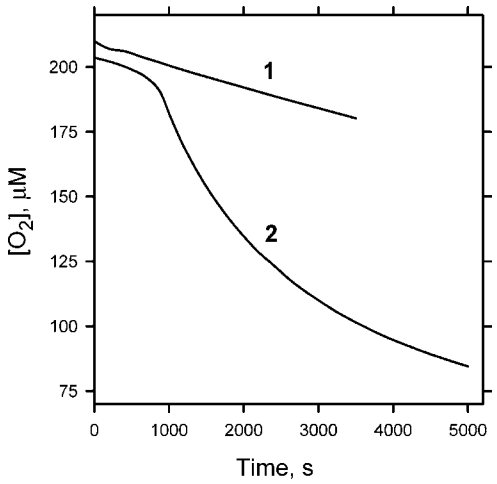

Figure 7. Kinetics of oxygen consumption accompanying the oxidation of 100 μM catechol at pH 8.70: trace 1, no SOD; trace 2, + 200 units/mL SOD.

When the process of oxygen consumption attained the end [$(R_{OX})_\infty \rightarrow 0$], evidently due to substrate depletion, it was possible to estimate the stoichiometric coefficient, *n*, that is, the number of oxygen molecules consumed per molecule of PP oxidized. These values are listed in **Table 1**. Only for the most oxidizable PG, EGC, and myricetin *n* could be determined at several pH values. For all of the other PP, the value of *n* could be measured in very alkaline medium (pH 13.0) only. Although *n* for PG was found to be almost the same, independent of pH, with EGC and myricetin *n* values increased significantly when the pH increased (**Table 1**). Among PP presented in **Table 1**, *n* values were previously reported at pH 14 for catechol (1.85), PG (1.15), catechin (1.85), and GA (2.15) (*16*). The comparison of the data reported in refer *16* with *n* values determined in this work (**Table 1**) shows that in some cases, especially for PG, our values of *n* are substantially higher than those reported in ref *16*. The reason for this discrepancy remains unknown; most likely this is caused by the difference in the experimental protocol. Elevated values of *n* for ECG and EGCG may be explained by the fact that these compounds contain both gallate and flavanol moieties; most likely, the *n* values for ECG and EGCG are the sum of those of the mentioned constituents.

The addition of SOD resulted in significant inhibition of the oxidation for all studied PP with the exception of catechol (see below) (**Figure 3**). The plot of R_{OX} versus SOD concentration tends to a limit that is reached typically at 20−40 units/mL SOD (not shown). The minimal values of $(R_{OX})_{SOD}/(R_{OX})$ characterizing the limiting effect of SOD are listed in **Table 1**. In contrast to other PP, for catechol the addition of SOD caused a significant increase (not a decrease) in R_{OX}. A specificity of the influence of SOD on catechol oxidation emerges also in the shape of $[O_2]$ traces under some conditions (nearly neutral pH and rather low SOD concentrations). In this case, the common shape (progressive decrease of R_{OX} with time) changes for an S-shaped trace (during the starting stage R_{OX} increased with time, and after going through the inflection point R_{OX} decreased, evidently due to substrate depletion) (**Figure 7**). It should be mentioned that similar S-shaped $[O_2]$ traces were previously reported during the oxidation of many *p*-hydroquinones and naphthoquinones (see ref *14* and references cited therein). It should be noted that the SOD effect for catechol does not flatten out even at 800 units/mL SOD (not shown), in contrast to all other PP studied.

Similar to tea extracts, adding milk resulted in the significant reduction of the starting value of R_{OX} for all of the studied PP. For instance, when 0.05 g/mL milk powder was added, the starting value of R_{OX} at pH 8.00 dropped from 142 to 25 nM/s during the oxidation of 100 μM EGC (**Figure 3**), from 65 to 15 nM/s for the oxidation of 200 μM GA, and from 150 to 38 nM/s for 100 μM EGCG.

DISCUSSION

Contribution of Individual Tea PP in the Oxidation of Tea Brew. This is determined by two factors: the quantity of individual PP in the extract and their oxidizability. The situation is more evident for green tea. Solid green tea contains 30−42% of various PP, basically catechins (see ref *17* and references cited therein). A significant portion of these phenolics is extracted in the process of brewing. In green tea extract, EGCG and ECG are the most abundant, and the amounts of EC and EGC are somewhat less. Other phenolics are present in rather minor concentrations. The typical quantities of main phenolic constituents in green tea brew reported in ref *9* (in milligrams per 1 g of leaves) are 78.1 (EGCG), 38.5 (ECG), 12.2 (EC), and 8.0 (EGC + catechin). By correlating these data with the oxidizability of PP presented in **Table 1**, one may conclude that the oxidation of EGCG makes the main contribution (~75%) to the oxidation of green tea extract; the contributions of ECG and EGC are ~10% each. The contribution of all other phenolics is almost immaterial. This finding is in line with the data of ref *9* reporting that incubation of green tea brew at pH 7.5 during 1 h at room temperature resulted in a loss of 95% EGCG.

As indicated above, the rate of the oxidation of black tea extracts is typically comparable with that of green tea extracts. The oxidizability of individual phenolic components of black tea has not been reported yet, and it is not easy to suggest individual compounds responsible for the oxidation of black tea extracts. Most likely, theaflavins, the main component of black tea phenolics (see ref *17* and references cited therein) are responsible for the consumption of oxygen by black tea extracts. Theaflavins have a rather low redox potential (*9*) and display very high reactivities toward superoxide (*18*) and peroxyl radicals (*19*). These properties of theaflavins allow the speculation that the oxidizability of theaflavins is also high.

Rate of Oxidation under Standard Conditions. Correlation between Oxidizability and PP Structure. Studies of the effects of pH and reactant concentrations on R_{OX} allow the extrapolation/interpolation of the starting rate of oxidation to the standard conditions, $(R_{OX})_{ST}$ (pH 7.40, 100 μM PP, 200 μM O_2). $(R_{OX})_{ST}$ may be considered as a measure of oxidizability. These data are listed in **Table 1**. Some problems arise when we estimate $(R_{OX})_{ST}$ for catechin, EC, and catechol. With catechin and EC, R_{OX} at pH <9 was too low to do the extrapolation directly. In these cases, $(R_{OX})_{ST}$ was estimated on the basis of R_{OX} measured at pH 9.0, assuming $\alpha = 0.85$ typical of other PP. As for catechol, the main problem is that R_{OX} increases very significantly in the presence of SOD (see above). The value of $(R_{OX})_{ST}$ for catechol presented in **Table 1** is related to the oxidation of catechol without SOD. Among other things, the data presented in **Table 1** allow the prediction of R_{OX} under any conditions.

The ratio of oxidizability $[(R_{OX})_{ST}]$ of model PP, catechol, GA, and PG is 1:23:140 (**Table 1**). Interestingly, the one-electron reduction potential E ($Q^{\bullet -}/QH_2$) decreases in the same direction: 447 mV for catechol (*21*), 391 mV for GA (*9*), and and 367 mV for PG (*20*). This is also true for the strengths of the O−H bond: 77.9 kcal/mol for catechol, 75.4 kcal/mol for

Table 2. Comparison between Kinetic Regularities of the Autoxidation of *p*-Hydroquinones and *o*-Hydroquinones (PP)

parameter	*p*-QH_2	PP (*o*-QH_2)
shape of $[O_2]$ traces	S-shaped	R_{OX} decreases with time
dependence of R_{OX} on $[QH_2]$	$R_{OX} \sim [QH_2]^2$	$R_{OX} \sim [QH_2]$
dependence of R_{OX} on $[O_2]$	independent	$R_{OX} \sim [O_2]$
stoichiometric factor *n*[a]	1	≥ 2

[a] Number of oxygen molecules consumed per molecule of PP oxidized.

GA, and 72.9 kcal/mol for PG (*21*). As for the oxidizability of natural PP, this is generally determined by the oxidizability of their key fragments. In particular, $(R_{OX})_{ST}$ for EGC, EGCG, and myricetin containing the pyrogallol fragment is close to the oxidizability of PG itself. $(R_{OX})_{ST}$ for ECG containing the gallate fragment almost coincides with that of GA. At the same time, the oxidizability of catechin and especially of EC is significantly lower than that of catechol (**Table 1**). A possible reason for that will be considered below.

Molecular Mechanism of Autoxidation of Tea PP. Main Factors Determining PP Oxidizability. By now the molecular mechanism of the oxidation of tea catechins (all of them are *o*-QH_2) is actually unknown in contrast to that of their *p*-analogues, *p*-QH_2 (*14*). To throw light on this problem, it seems rational to compare kinetic regularities of the autoxidation of *o*-QH_2 with those of *p*-QH_2 (**Table 2**). Although the kinetic regularities for *o*-QH_2 were elucidated during this study, those for *p*-QH_2 are taken mostly from previous work (*14*). It may be seen that there is a dramatic difference between *o*-QH_2 and *p*-QH_2. These two kinds of QH_2 differ also in absolute rates of oxidation; in most cases the oxidizability of *p*-QH_2 is higher under comparable conditions.

The autoxidation of *p*-QH_2 was found to be a self-accelerated, autocatalytic process catalyzed by a corresponding quinone (*p*-Q), the product of *p*-QH_2 oxidation (*14*). In the simplest form, the process may be presented by the following scheme:

$$Q + QH_2 \rightarrow 2Q^{\bullet -} + 2H^+ \quad (1)$$

$$Q^{\bullet -} + O_2 \leftrightarrow Q + O_2^{\bullet -} \quad (2)$$

$$O_2^{\bullet -} + QH_2 \rightarrow Q^{\bullet -} + H_2O_2 \quad (3)$$

The oxidation is initiated by reaction 1. The most significant features of *p*-QH_2 are S-shaped $[O_2]$ traces and independence of R_{OX} of $[O_2]$; these were not only observed experimentally but also confirmed by computer simulation performed on the basis of the above scheme (*14*). Because the kinetic regularities of the oxidation of *o*-QH_2 differ dramatically from those of *p*-QH_2, we should conclude that in the case of *o*-QH_2 the process is not initiated by reaction 1.

The idea that the oxidation of phenolics may be catalyzed by transition metals is rather popular among researchers. Precautions have been taken to reduce this effect in our work. First of all, this was provided by the high quality of phosphates applied to prepare buffers, as Merck-specified salts contained very low concentrations of transition metals (<0.01 ppm of Cu and <0.05 ppm of Fe). This is also due to additional treating solutions of individual phosphates used to prepare buffers by Chelex; the phosphate buffers applied satisfied Buettner's test (low rate of ascorbate oxidation in buffer tested) (*22*). Therefore, we had no reason to expect any significant catalytic effect of transition metals, in particular, that of Fe(III) or Cu(II) on the oxidation of PP in our experiments. The latter is also evident from the fact that the known chelating agents, EDTA, supressing the catalytic activity of Cu(II), and Desferal, which supresses the catalytic activity of both Fe(III) and Cu (II) (*23*), had no effect on the kinetics of PP oxidation (not shown). The same finding was also reported in ref *11*.

It remains to suggest that the autoxidation of *o*-QH_2 is initiated by the reaction of molecular oxygen with substrate.

$$QH_2 + O_2 \rightarrow QH^{\bullet}\,(Q^{\bullet -}) + HO_2^{\bullet}\,(O_2^{\bullet -} + 2H^+) \quad (4)$$

The latter is in line with the fact that R_{OX} for PP is nearly proportional to oxygen concentration (**Figure 5**). An additional argument for the key role of reaction 4 is that the oxidizability of PP correlates with E ($Q^{\bullet -}/QH_2$) (see above) rather than with the difference E ($Q/Q^{\bullet -}$) $-$ E ($Q^{\bullet -}/QH_2$), as this was reported for the autoxidation of *p*-QH_2 (*13*).

Reaction 4 is expected to be very slow as it is thermodynamically highly unfavorable. The enthalpy of reaction 4 may be calculated as the difference between the strength of the O$-$H bond in *o*-QH_2 [$\sim$80 kcal/mol (*21*)] and that in $HO_2^{\bullet}$ [48 kcal/mol (*24*)]. Thus, reaction 4 is highly endothermic and the expected rate constants at pH 7.4 hardly exceed 1 M^{-1} s^{-1}. Meanwhile, rate constants for reaction 1 for all of the studied *p*-QH_2 at pH 7.4 are significantly higher [for example, 1400 M^{-1} s^{-1} for nonsubstituted *p*-QH_2 (*25*)]. This is evidently the main reason the oxidation of *o*-QH_2 initiated by reaction 4 occurs more slowly than the oxidation of *p*-QH_2, with pH being the same. At higher pH, reaction 4 may also occur with participation of deprotonated forms of *o*-QH_2. In this case, reaction 4 becomes less endothermic; this is expected to decrease the activation energy and hence increase the rate of reaction 4. Thus, the rate of the oxidation of *o*-QH2 should be accelerated at higher pH as observed experimentally (**Figure 6**).

The question arises: why does reaction 1, the key reaction for the autoxidation of *p*-QH_2, not contribute in the case of the autoxidation of PP (*o*-QH_2)? Most likely, a reason for that is the much lower stability of *o*-quinones in aqueous environment as compared to *p*-quinones. *o*-Quinones have been reported to be very unstable; they readily participate in nucleophilic reactions with water and other substrates including *o*-QH_2 (see ref *26* and references cited therein). As a result, the steady-state concentration of *o*-quinones and hence the rate of reaction 1 are rather low. Catechol seems to be intermediate between *p*-QH_2 and all other *o*-QH_2. As mentioned above, under some conditions, catechol oxidizes with pronounced self-acceleration, suggesting that reaction 1 does occur, at least to some extent. Besides, catechol oxidizes significantly more quickly than catechin and EC, also containing a catechol moiety (**Table 1**). Most likely, *o*-quinones derived from catechin and EC are less stable than that derived from catechol itself.

In contrast to *p*-QH_2 a kinetic scheme for PP autoxidation cannot be presented in detail. To the first approximation, this may be presented by reactions 2$-$4. Generally, this is a chain process with $O_2^{\bullet -}$ as a chain-carrying free radical. The latter follows from the fact that SOD affects R_{OX}. Typically, the addition of SOD inhibits PP oxidation (**Table 1**; **Figure 3**), but in the case of catechol, SOD shows the pronounced promoting effect (**Figure 7**; **Table 1**). In principle, SOD may play two roles during the autoxidation of hydroquinones (*14*). On the one hand, purging the system of $O_2^{\bullet -}$ inhibits reaction 3 and thus decreases the rate of the total process. On the other hand, the decrease in the steady-state concentration of $O_2^{\bullet -}$ can shift the equilibrium (2) to the right. For *p*-QH_2 the net effect is basically determined by the one-electron reduction potential E ($Q/Q^{\bullet -}$). When E ($Q/Q^{\bullet -}$) is more positive than E ($O_2/O_2^{\bullet -}$) $= -155$

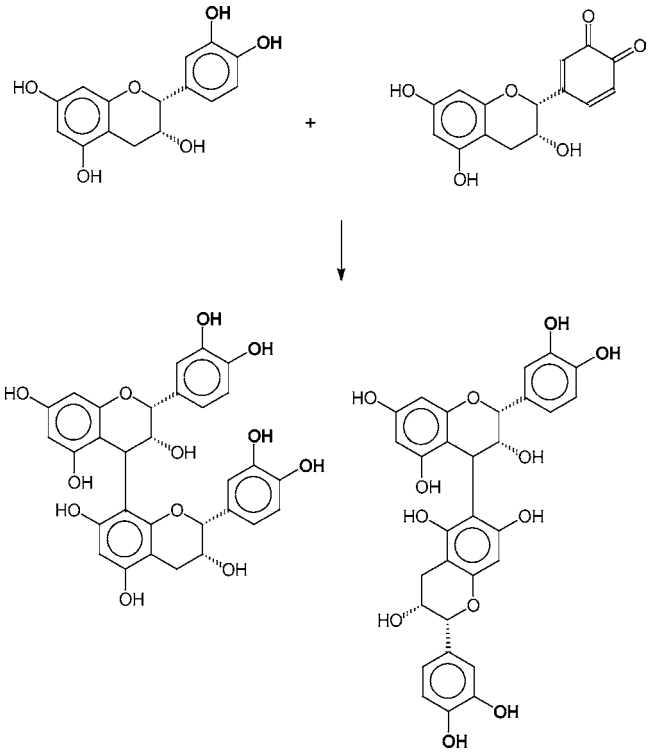

Figure 8. Dimerization of epicatechin.

mV (at pH 7.0), the addition of SOD stimulates the oxidation; when E ($Q/Q^{\bullet-}$) is more negative than −155 mV, adding SOD results in inhibition. The value of E ($Q/Q^{\bullet-}$) for catechol is around +300 mV (estimated on the basis of data presented in ref *25*), which is much more positive than E ($O_2/O_2^{\bullet-}$). This correlates well with the promoting effect of SOD on catechol oxidation. The above consideration is valid provided that Q is stable enough to participate in equilibrium 2. Most likely, this is not true for quinones derived from all other PP. This seems to be the reason SOD shows an inhibiting effect on the oxidation of PP different from that of catechol. As for catechin and EC, which contain a catechol fragment, E ($Q/Q^{\bullet-}$) hardly differs significantly from that of catechol itself.

The next question is: Why does the stoichiometric coefficient n for the oxidation of all the studied PP exceed 1 (**Table 1**), in contrast to *p*-hydroquinones, for which n is always around 1 (*14*)?. The elevated value of n for PP may be assigned to the contribution of some products of PP oxidative transformation. For catechins, dimers/oligomers are most likely such products. Dimers have been repeatedly reported among products of PP oxidative transformations (*14*, *27*−*29*). As for the pathway for PP dimerization, this is most likely the Michael-type addition of *o*-quinone, a primary product of PP transformation, to the original PP (see ref *29* and references cited therein). This is exemplified in **Figure 8** for epicatechin. A remarkable feature of such PP dimers/oligomers is that they contain the same number of active OH groups (on monomer unit basis) as the original compound; these OH groups are likely able to undergo further oxidation. When PP is completely converted into a dimer, the expected value of n is 2. The value of n will be even higher if further oligomerization occurs. A similar mechanism has been suggested to explain the elevated stoichiometric coefficients of inhibition, when catechins and some other PP are applied as chain-breaking antioxidants (*15*).

Some Practical Consequences. Let us estimate how the autoxidation of tea phenolics occurs in the course of tea brewing and then in the body after the consumption of tea brew. Freshly boiling water applied to brew tea is evidently almost free of oxygen; this means that despite the rather high temperature, the oxidation at the first moment of placing tea leaves into boiling water occurs slowly. The oxidation becomes faster when oxygen diffuses into brew, especially if it undergoes agitation. By the moment when tea brew is ready for drinking (in a few minutes), some portion of tea PP, first of all, the most oxidizable constituents, EGC and EGCG in green tea and theaflavins in black tea, have been lost, being converted into products. The latter is accompanied by the formation of H_2O_2 (*8*, *12*). As it follows from **Figure 2**, the degree of advancement of the reaction under common conditions of brewing is likely not too significant (not more than 10−15%). As soon as tea extract is consumed and goes to the stomach, tea oxidation is almost suspended as the pH in the stomach is low, ∼3. When tea phenolics go to the intestines, where the pH is ∼7.4, the oxidation can resume. However, this process is partly inhibited by SOD (see above).

As mentioned above, milk addition caused the pronounced inhibition of tea brew oxidation (**Figure 2**). To some extent this is associated with the influence of milk on the antioxidative activity of tea brew and tea PP; this problem has received some attention in the literature (*9*, *30*, *31*). The inhibiting effect of milk on the antioxidant activity of tea phenolics is commonly explained by binding milk proteins to PP (*32*). A similar idea may be also exploited to explain the inhibiting effect of milk on tea brew oxidation; it is possible to speculate that PP species bound to milk proteins are less accessible for oxidation than free PP species. It should be noted that although SOD addition causes a rather moderate effect on the antioxidant activity, the effect of milk is much more pronounced. Independent of the mechanism underlying the inhibiting effect of milk on the autoxidation of tea brew, the latter may be considered as the "theoretical basis" of the English tradition to drink strong tea, but with the addition of milk. The other popular tradition of drinking tea with added lemon has a similar "theoretical basis": first, the decrease of pH results in a decrease in the rate of the autoxidation of tea brew; second, ascorbic acid, which is abundant in lemon, was reported to be an effective inhibitor of PP oxidation (*10*, *33*).

ABBREVIATIONS USED

PP, polyphenol; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; PG, pyrogallol; GA, gallic acid; SOD, superoxide dismutase; QH_2, hydroquinone; Q, quinone; $Q^{\bullet-}$, semiquinone; R_{OX}, rate of oxidation; $(R_{OX})_{ST}$, rate of oxidation under standard conditions.

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