STUDIES OF THE EFFECTS OF CATECHIN ISOMERS ON OXYMYOGLOBIN OXIDATION AND LIPID OXIDATION IN MUSCLE MODEL SYSTEMS

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Introduction

Catechins are the major group of polyphenolic flavonoids found in tea. In addition to reported health benefits, tea catechins are potent antioxidants and activity has been reported in a variety of test systems (He and Shahidi, 1997; Huang and Frankel, 1997). Polyphenolic compounds have also been shown to react with proteins for example, the muscle pigment myoglobin (Kroll and Rawel, 2001). The principle catechins found in green tea (*Camellia sinensis*) are (–)-epicatechin (–EC), (–)-epigallocatechin (–EGC), (–)-epicatechin gallate (–ECG) and (–)-epigallocatechin gallate (–EGCG). Structural differences between tea catechin isomers result in varying degrees of antioxidant potency. –EC has an ortho-dihydroxyl group on the B-ring at carbons 3 and 4 and a hydroxyl group at carbon 3 on the C ring. –EGC differs from –EC in that it has a tri-hydroxyl group at carbons 3, 4, and 5 on the B ring. –ECG has a gallate moiety esterified at carbon 3 on the C ring. –EGCG has both a tri-hydroxyl group at carbons 3, 4, and 5 on the B ring and a gallate moiety esterified at carbon 3 on the C ring (Higdon and Frei, 2003). Studies within our research group have focused on the enhancement of fresh beef quality through supplementation of animal diets with green tea catechins. Direct addition of the tea catechin supplement to minced beef resulted in greater colour and lipid stability (Maher et al., 2002). Following HPLC separation (Tsuchiya et al., 1998), the tea catechin supplement, added to animal diets or directly to minced beef, contained approximately 8% –EC, 10% –EGC, 18% –ECG, and 44% EGCG. The influence of individual catechin isomers on oxymyoglobin (oxyMb) oxidation and lipid oxidation in muscle based model systems, at a temperature (4ºC) and pH (5.5) relevant to fresh beef in storage, merits investigation.

Objectives

The objective of this study was to examine the influence of individual catechin isomers (+C, –EC, –EGC, –ECG, and –EGCG) on oxyMb (horse heart) oxidation over time. The influence of catechin isomers on oxyMb and lipid oxidation in 25% *M. longissimus dorsi* (LD) homogenates was also investigated.

Methodology

Horse heart oxyMb was prepared according to a modification of the method of Brown and Mebine (1969). Incubates (3 ml) containing oxyMb (~ 1 mg/ml) and catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) at concentrations of 50, 25, 12.5, 6.25 and 3.125 μM in 150 mM KH2PO4-KOH, pH 5.5 were prepared. Methanol was used to dissolve catechin isomers and the methanol content of incubates was 5%. Incubates without catechin isomers containing methanol were run simultaneously as controls. Incubates were stored at 4°C and oxyMb oxidation was monitored over a 5 day storage period.

M. Longissimus dorsi (LD) homogenates (25%) were prepared in 0.12 M KCl 5mM histidine buffer using an Ultra Turrax tissue homogenizer. Lipid oxidation in 30 ml LD homogenate samples, held at 4°C, was initiated by the addition of 45 μM FeCl3/sodium ascorbate (1:1). Catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) were added to LD homogenates at concentrations of 250, 100 and 50 μM. Catechin isomers were dissolved in methanol and the final methanol content of LD homogenates was 5%. LD homogenates with methanol and without FeCl3/ascorbate were run simultaneously as controls. Lipid oxidation and oxyMb oxidation was measured in samples held at 4°C for up to 24 hours.

Lipid oxidation in LD homogenates was measured following a modification of the 2-thiobarbituric acid-reactive substances (TBARS) procedure of Siu and Draper (1978). The protein content of the LD homogenates was determined according to the method of Markwell et al. (1978). Lipid oxidation was expressed as TBARS in nmoles malondialdehyde (MDA)/mg protein.

OxyMb oxidation in incubates containing horse heart oxyMb was measured by directly recording the absorbance at selected wavelengths. Spectral scans were recorded by scanning from 730 to 500 nm.

OxyMb oxidation in 25% LD homogenates was measured in the supernatant obtained after centrifuging 5 ml of LD homogenate at 14,000 g for 15 min at 4°C. The supernatant was subsequently filtered through Whatman 541 filter paper and re-centrifuged at 14,000 g for 15 min at 4°C.

The relative proportion of oxyMb (% of total myoglobin) in incubates containing horse heart oxyMb and in LD homogenates was calculated using absorbance measurements at selected wavelengths (572, 565, 545 and 525 nm) as described by Krzywicki (1982).

Each experiment was performed three times and all analysis was carried out in duplicate. Data was analysed using the SPSS (version 11.0) statistical package.

Results & Discussion

Catechin isomers exhibited varying degrees of pro-oxidant activity on horse heart oxyMb oxidation (Table 1). Catechins +C and its epimer –EC did not enhance oxyMb oxidation over the 5 day storage period. –EGC exhibited greater pro-oxidant activity, compared to +C and –EC, on oxyMb oxidation and, in general, oxyMb oxidation increased with increasing catechin concentration. Catechins containing gallate moieties (–ECG and –EGCG) exhibited the greatest pro-oxidant effects on oxyMb oxidation

(Table 1). The reactivity of plant phenolic compounds with myoglobin is dependent on the number and position of phenolic hydroxyl groups (Kroll and Rawel, 2001). In the present study, the pro-oxidant activity of catechin isomers on oxyMb oxidation increased proportionally with the number of hydroxyl groups. The exact mechanism by which catechin isomers promote oxyMb oxidation is unclear. Flavonoids are known to bind with proteins (Arts et al., 2002) and as such, it is possible that the different catechin isomers bind or interact with oxyMb in ways which render the pigment susceptible to oxidation. No oxyMb spectral shift was observed in the presence of 50 µM –EGCG indicating lack of an interaction between catechin molecules and oxyMb (Figure 1). Nakayama et al. (2002) reported that hydrogen peroxide (H_2O_2) is formed in aqueous solutions of tea catechins where –EGC and –EGCG contribute mainly to H_2O_2 formation. Since hydrogen peroxide is a potent catalyst of oxyMb oxidation, this may, in part explain the pro-oxidative nature of –EGC and –EGCG on oxyMb.

Addition of $FeCl_3$/ascorbate (45 µM) resulted in increased lipid oxidation in LD homogenates over the storage period (Table 2). Graded addition of each catechin isomer ($p < 0.05$) reduced lipid oxidation compared to controls after 24 hours storage at 4ºC. Lipid oxidation decreased with increasing concentrations of +C and –EC. Catechins, –EGC, –ECG and –EGCG exhibited similar antioxidant activity irrespective of catechin concentration. In addition, catechins containing gallate groups (–ECG and –EGCG) exerted the greatest antioxidant activity compared to other catechin isomers. Guo et al. (1999) suggested that the presence of the gallate group played the most important role in free radical scavenging ability of catechins, and that the additional hydroxyl group on –EGC also contributed to radical scavenging activity. OxyMb oxidation was reduced ($p < 0.05$) in the presence of each catechin isomer (Table 2). Catechin isomers did not enhance oxyMb oxidation in LD homogenates in contrast to the pro-oxidant activity of catechins on horse heart oxyMb reported above. This may be attributed to catechins having greater affinity for other components in LD homogenates such as other proteins or lipids.

Conclusions

Catechins did not delay horse heart oxyMb oxidation and isomers containing gallate groups exerted the greatest pro-oxidant activity. Catechins reduced lipid oxidation in LD homogenates and isomers containing gallate groups had the greatest antioxidant activity. Results indicate differing reactivity of catechin isomers with muscle lipids and oxyMb depending on the test system employed.

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Tables and Figures

Table 1. OxyMb oxidation following the addition (50, 25, 12.5, 6.25 and 3.125 μM) of catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) and storage at 4°C.

Incubate	μM	Time, days					
		0	1	2	3	4	5
OxyMb[1]		96.0 ± 4.0	83.4 ± 2.1	69.2 ± 4.0	58.2 ± 3.4	48.5 ± 4.5	41.8 ± 5.1
OxyMb +							
+C	50	96.6 ± 2.5^{a}	79.3 ± 5.3^{a}	61.5 ± 4.5^{a}	49.7 ± 4.6^{a}	39.1 ± 3.5^{a}	31.1 ± 2.0^{a}
	25	96.4 ± 2.3^{a}	78.9 ± 3.2^{a}	60.9 ± 3.9^{a}	49.2 ± 3.4^{a}	38.4 ± 2.8^{a}	31.1 ± 3.2^{a}
	12.5	96.2 ± 2.6^{a}	81.0 ± 5.9^{a}	65.0 ± 5.4^{a}	53.1 ± 5.4^{a}	42.6 ± 4.4^{a}	36.0 ± 4.7^{a}
	6.25	95.9 ± 3.0^{a}	81.2 ± 5.7^{a}	65.4 ± 4.8^{a}	53.7 ± 5.2^{a}	43.6 ± 4.4^{a}	36.3 ± 4.1^{a}
	3.125	95.4 ± 2.9^{a}	79.0 ± 8.9^{a}	62.4 ± 9.4^{a}	51.0 ± 10.0^{a}	41.2 ± 9.0^{a}	34.3 ± 8.9^{a}
–EC	50	94.8 ± 3.9^{a}	78.7 ± 6.7^{a}	61.6 ± 7.0^{a}	49.0 ± 8.5^{a}	39.1 ± 8.8^{a}	31.8 ± 8.5^{a}
	25	94.8 ± 3.2^{a}	78.5 ± 7.4^{a}	62.7 ± 9.4^{a}	50.7 ± 10.0^{a}	40.8 ± 9.2^{a}	33.3 ± 10.0^{a}
	12.5	93.5 ± 3.6^{a}	79.6 ± 5.6^{a}	64.4 ± 6.5^{a}	53.6 ± 7.7^{a}	43.6 ± 7.4^{a}	36.7 ± 7.1^{a}
	6.25	95.2 ± 3.3^{a}	82.1 ± 2.4^{a}	67.4 ± 3.0^{a}	57.1 ± 3.5^{a}	47.2 ± 3.8^{a}	39.9 ± 3.4^{a}
	3.125	94.8 ± 3.7^{a}	80.7 ± 2.6^{a}	65.2 ± 3.3^{a}	54.7 ± 2.4^{a}	44.6 ± 1.7^{a}	37.3 ± 1.7^{a}
–EGC	50	91.9 ± 5.8^{a}	70.8 ± 6.7^{a}	51.2 ± 6.2^{a}	38.4 ± 6.2^{a}	28.7 ± 4.8^{ab}	22.6 ± 3.8^{ab}
	25	91.3 ± 6.5^{a}	67.9 ± 9.4^{a}	46.1 ± 9.9^{a}	33.1 ± 9.3^{a}	24.0 ± 7.6^{a}	18.5 ± 6.4^{a}
	12.5	91.6 ± 5.4^{a}	72.7 ± 7.9^{a}	55.4 ± 8.7^{a}	43.6 ± 9.2^{a}	34.5 ± 8.0^{ab}	28.2 ± 7.1^{ab}
	6.25	91.9 ± 5.8^{a}	75.7 ± 9.5^{a}	60.5 ± 10.0^{a}	50.1 ± 9.9^{a}	41.1 ± 9.5^{ab}	34.8 ± 9.7^{ab}
	3.125	93.5 ± 5.3^{a}	79.0 ± 4.8^{a}	64.7 ± 5.1^{a}	54.7 ± 5.9^{a}	45.7 ± 5.7^{b}	39.5 ± 4.9^{b}
–ECG	50	86.0 ± 8.2^{a}	50.7 ± 5.7^{a}	33.3 ± 3.9^{a}	23.4 ± 2.9^{a}	16.7 ± 2.1^{a}	12.4 ± 1.4^{a}
	25	87.7 ± 7.8^{a}	55.6 ± 3.0^{a}	38.2 ± 3.0^{ac}	27.8 ± 2.3^{ac}	19.6 ± 1.9^{a}	14.2 ± 1.7^{ac}
	12.5	90.2 ± 5.6^{a}	61.8 ± 6.3^{ab}	44.1 ± 4.7^{ad}	33.3 ± 4.6^{ad}	24.4 ± 3.1^{ac}	18.0 ± 2.5^{ad}
	6.25	88.8 ± 5.6^{a}	66.4 ± 9.0^{ac}	50.3 ± 7.7^{bcd}	39.2 ± 6.9^{bcd}	29.8 ± 5.1^{bc}	22.0 ± 3.7^{bcd}
	3.125	91.9 ± 5.7^{a}	73.9 ± 4.5^{bc}	57.9 ± 5.1^{b}	46.6 ± 5.4^{b}	35.6 ± 5.3^{b}	27.0 ± 4.5^{b}
–EGCG	50	78.8 ± 9.4^{a}	38.0 ± 8.8^{a}	22.7 ± 5.7^{a}	15.4 ± 4.6^{a}	11.4 ± 2.7^{a}	8.4 ± 2.0^{a}
	25	84.9 ± 7.8^{a}	44.3 ± 8.4^{ac}	26.1 ± 5.3^{a}	17.9 ± 3.7^{a}	12.5 ± 2.4^{a}	9.3 ± 1.8^{a}
	12.5	88.7 ± 7.7^{a}	51.4 ± 6.4^{ad}	30.1 ± 2.5^{a}	22.4 ± 2.5^{a}	16.6 ± 1.7^{a}	12.4 ± 0.8^{a}
	6.25	90.7 ± 6.0^{a}	62.8 ± 7.6^{bcd}	45.4 ± 6.0^{b}	33.0 ± 9.8^{ac}	28.7 ± 5.0^{b}	23.5 ± 3.9^{b}
	3.125	91.2 ± 6.0^{a}	69.5 ± 7.4^{bd}	54.3 ± 7.7^{b}	44.8 ± 7.9^{bc}	35.9 ± 6.1^{b}	31.0 ± 7.2^{b}

1Oxymyoglobin, % of total, control containing 5% methanol. abcdMean values for the same catechin group within each day bearing different superscripts are significantly different, $P < 0.05$.

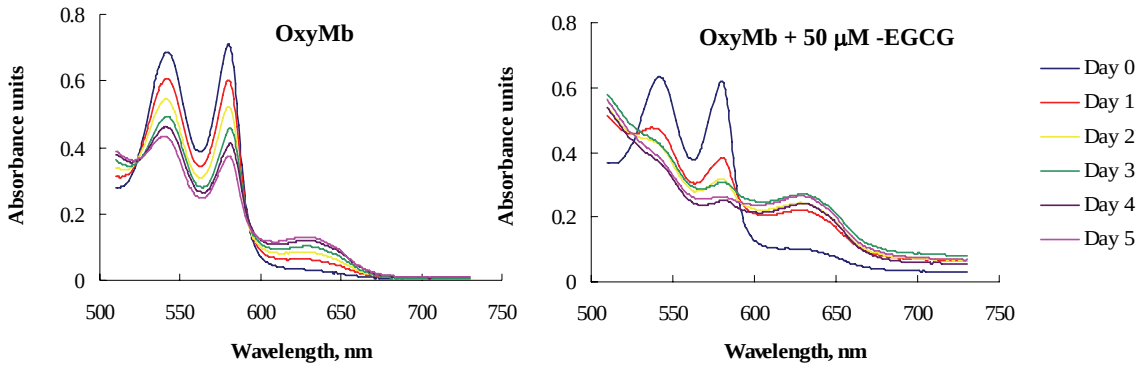

Figure 1. Absorbance spectra of oxyMb alone or oxyMb in the presence of 50 µM –EGCG.

Table 2. OxyMb oxidation and lipid oxidation in 25% M. *longissimus dorsi* homogenates following the addition (250, 100 and 50 µM) of catechin isomers (+C, –EC, –EGC, –ECG and –EGCG) and storage at 4°C.

Incubate	µM	Time, hours			
		0		24	
		[1]OxyMb, %	[2]TBARS	OxyMb, %	TBARS
H[3]		87.6 ± 10.8[a]	0.008 ± 0.005[a]	83.8 ± 5.7[a]	0.021 ± 0.012[a]
H + P[4]		84.6 ± 12.4[a]	0.010 ± 0.003[a]	51.2 ± 6.8[b]	0.264 ± 0.043[b]
H + P +					
+C	250	86.4 ± 10.9[a]	0.009 ± 0.005[a]	78.2 ± 5.5[a]	0.014 ± 0.008[a]
	100	86.7 ± 10.5[a]	0.010 ± 0.006[a]	77.4 ± 5.0[a]	0.053 ± 0.036[ad]
	50	87.5 ± 9.7[a]	0.009 ± 0.004[a]	75.2 ± 3.7[a]	0.115 ± 0.049[cd]
–EC	250	85.9 ± 10.7[a]	0.008 ± 0.004[a]	76.4 ± 2.3[a]	0.012 ± 0.011[a]
	100	86.6 ± 10.9[a]	0.011 ± 0.004[a]	78.3 ± 5.1[a]	0.025 ± 0.016[ad]
	50	86.9 ± 11.0[a]	0.015 ± 0.011[a]	75.4 ± 4.1[a]	0.085 ± 0.038[cd]
–EGC	250	86.3 ± 10.7[a]	0.009 ± 0.002[a]	78.7 ± 5.4[a]	0.009 ± 0.007[a]
	100	86.8 ± 9.6[a]	0.010 ± 0.002[a]	78.9 ± 5.6[a]	0.014 ± 0.009[a]
	50	86.4 ± 10.6[a]	0.008 ± 0.005[a]	76.2 ± 3.0[a]	0.024 ± 0.009[a]
–ECG	250	85.7 ± 10.5[a]	0.008 ± 0.005[a]	79.3 ± 5.9[a]	0.010 ± 0.008[a]
	100	85.8 ± 10.1[a]	0.010 ± 0.003[a]	79.5 ± 6.2[a]	0.008 ± 0.007[a]
	50	84.8 ± 11.2[a]	0.009 ± 0.002[a]	78.4 ± 5.9[a]	0.017 ± 0.011[a]
–EGCG	250	86.4 ± 10.0[a]	0.010 ± 0.003[a]	80.1 ± 5.7[a]	0.007 ± 0.006[a]
	100	87.1 ± 10.4[a]	0.009 ± 0.003[a]	80.3 ± 5.7[a]	0.006 ± 0.005[a]
	50	86.5 ± 9.4[a]	0.010 ± 0.002[a]	80.1 ± 5.6[a]	0.011 ± 0.008[a]

1Oxymyoglobin, % of total. 2nmoles malondialdehyde/mg protein. 325% LD homogenate + 5% methanol. 4FeCl3/ascorbate. abcdMean values within the same catechin group (and compared to controls H, H + P) bearing different superscripts are significantly different, $P < 0.05$.