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Exploring the Potential Effect of Phospholipase A2 Antibody to Extend Beef Shelf-Life in a Beef Liposome Model System

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Exploring the Potential Effect of Phospholipase A2 Antibody to Extend Beef Shelf-Life in a Beef Liposome Model System

C.K.Y. Chun, R. Welte, M. Roth, M.P. Richards,¹ and M.D. Chao

Abstract

The objective of this study was to utilize a beef liposome model system to investigate if phospholipase A2 antibody (aPLA2) can be used to inhibit phospholipase A2 (PLA2) activity to potentially improve beef shelf-life. The phospholipid (PL) from 10 steaks were split into six different treatments in a 2.5 mL buffer solution: 1) PL (25 mg of PL); 2) aPLA10 (PL + 25 µg of aPLA2); 3) aPLA20 (PL + 50 µg of aPLA2); 4) PLA2 (PL + 10 µg of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25 µg of aPLA2); and 6) PLA2+aPLA20 (PL + PLA2 + 50 µg of aPLA2). An aliquot was taken from each of the 6 treatments for PL profile analysis and product ion analysis by mass spectrometry. Eighty µM of bovine myoglobin was added to the remaining samples and exposed to retail display conditions (39°F; 2300 lux) for 7 days. At day 0, 1, 4, and 7, aliquots were taken for lipid oxidation analysis. There was a display × treatment interaction ($P < 0.01$) for lipid oxidation. At day 7 of display, PLA2, PLA2+aPLA10, and PLA2+aPLA20 treatments had greater ($P < 0.01$) lipid oxidation compared to the samples without PLA2. This trend was seen in the other retail display periods. The PL profile analysis showed clear differences between treatments with or without PLA2. The PLA2 treatments showed greater ($P < 0.01$) relative percent of total lysophosphatidylcholine (LysoPC) than treatments without PLA2. The PLA2 treatments had less ($P < 0.05$) relative percent of total ether-linked phosphatidylcholine (ePC) than treatments without PLA2, specifically, ePC 34:1, 34:2, 34:4, 36:1, 36:3, and 36:4. Finally, it appeared that aPLA2 had no effect on inhibiting PLA2 hydrolysis as there was no difference ($P > 0.10$) between PLA2 and aPLA+PLA2 treatments in relative percent of total ePC, phosphatidylcholine (PC) as well in LysoPC composition.

Introduction

Phospholipase-A₂ (PLA2) is the ubiquitous enzyme that cleaves a fatty acid tail at the sn-2 position from a phosphatidylcholine (PC), an abundant phospholipid (PL) class in cell membranes. The resulting free fatty acids (FFA) are typically polyunsaturated fatty acids (PUFA) which are prone to lipid oxidation when exposed to pro-oxidants such as light and oxygen. A PLA2 antibody (aPLA2) can be mass-produced through laying hens, and the egg powder containing aPLA2 has been used as a feed supplement to improve growth performance for various livestock. We hypothesized that the aPLA2 from egg powder may inhibit PLA2 activity, thus preventing the formation of FFA,

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which can potentially improve the shelf stability of beef. Therefore, the objective of this study was to utilize a beef liposome model system to investigate this proposed mechanism.

Experimental Procedures

Total lipid was extracted from 10 U.S. Department of Agriculture choice beef loins at 3 days post-mortem via chloroform, methanol, and water. Phospholipids were separated from each lipid pool using solid phase extraction with amino propyl silica cartridges. Solvents were evaporated under vacuum and nitrogen gas. Phospholipids from each steak were further split into six different treatments: 1) PL (25 mg of PL); 2) aPLA10 (PL + 25µg of aPLA2); 3) aPLA20 (PL + 50µg of aPLA2); 4) PLA2 (PL + 10µg of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25µg of aPLA2); 6) PLA2+aPLA20 (PL + PLA2 + 50 µg of aPLA2). Phospholipase A2 antibody was extracted from hyperimmunized eggs, and porcine pancreatic PLA2 was used. Treatments were mixed with a tris/CaCl₂ buffer (pH 8.0) and incubated at 98°F for 2 hours to activate PLA2. After incubation, an aliquot was immediately taken for PL profile analysis and product ion analysis by mass spectrometry. Eighty µM of bovine myoglobin was added to the remaining model system and exposed to retail display conditions (39°F; 2300 lux) for 7 days. At days 0, 1, 4, and 7, aliquots were taken for lipid oxidation analysis as determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) and calculated as mg of malondialdehyde (MDA) per mg of phospholipid.

Results and Discussion

As expected, the PL profile analysis showed clear differences between treatments with or without PLA2. The PLA2 treatments showed greater relative percent of total lyso-phosphatidylcholine (LysoPC) than treatments without PLA2 (Figure 1; $P < 0.01$). Again, in PLA2 treatments individual LysoPC species 16:0, 16:1, 18:0, and 18:1 had higher relative percent than treatments without PLA2 (Figure 2; $P < 0.01$). The PLA2 treatments had significantly less relative percent of total ePC than treatments without PLA2 (Figure 1), specifically, ePC 36:1, 36:2, 36:3, 36:4, and 36:5 (Figure 3; $P < 0.05$). Also, in phosphatidylethanolamine (PE), PLA2 treatments had significantly less relative percent than treatments without PLA2 (Figure 1; $P < 0.01$). Interestingly, the PLA2 treatments did not seem to have significant effect on relative percent of total PC as seen in ePC as treatments were not significantly different (Figure 1; $P > 0.10$). It appears that aPLA2 had no effect on inhibiting PLA2 hydrolysis as there was no difference between PLA2 and PLA2+aPLA treatments in relative percent of total ePC, PC, or in PL degradation products ($P > 0.10$). Product ion analysis revealed for ePC species the major fatty acid combinations consisted of 18:0/18:1, 18:0/18:2, 18:1/18:2, and 18:2/18:2 (Table 1). There was a display × treatment interaction for lipid oxidation (Figure 5; $P < 0.01$). At 7 days of display, PLA2, PLA2+aPLA10, and PLA2+aPLA20 treatments had greater lipid oxidation than treatments without PLA2 added ($P < 0.01$). A similar trend was seen within earlier display days as well. At 4 days, PLA2+aPLA20 had less oxidation than 4-day PLA2 ($P < 0.05$). Interestingly, 7-day aPLA10 and aPLA20 had less lipid oxidation than 7-day PL and less oxidation than 4-day PLA2 ($P < 0.05$), potentially through an oxidation effect. This study confirms that PLA2 significantly alters PL composition and that the hydrolysis of PL by PLA2 influences lipid oxidation. Although no inhibition effect was observed for PLA2 by aPLA2, there appears to be an antioxidant effect for aPLA2 for lipid oxidation. However, PLA2

appears to attack ePC more effectively than PC and shows a preference for hydrolyzing PL containing 18:2 over 20:4.

Implications

Phospholipase A2 significantly alters beef phospholipids composition, making it more susceptible to lipid oxidation. Although no inhibition effect was observed for PLA2 by aPLA2, there appears to be an antioxidant effect for aPLA2 for lipid oxidation.

Acknowledgments

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Table 1. Likely combinations of fatty acids identified

Apparent lipid molecular species (total acyl carbons:total double bonds)	Fatty acid combinations identified	Relative abundance (%)
ePC ¹		
36:1	18:0/18:1	54.64
36:2	18:0/18:2	46.48
	18:1/18:1	37.82
36:3	18:1/18:2	78.13
	16:0/20:3	9.51
36:4	18:2/18:2	46.10
	16:1/20:3	22.73
	16:0/20:4	21.60
36:5	16:1/20:4	78.69
PC ²		
36:1	18:0/18:1	81.70
36:2	18:1/18:1	47.22
	18:0/18:2	43.62
	16:0/20:2	3.80
36:3	18:1/18:2	83.39
	16:0/20:3	10.56
	18:0/18:3	6.05
36:4	16:0/20:4	65.32
	18:2/18:2	19.00
	18:1/18:3	7.9
36:5	16:1/20:4	69.36
	16:0/20:5	18.13

¹ePC = ether-linked phosphatidylcholine.

²PC = phosphatidylcholine.

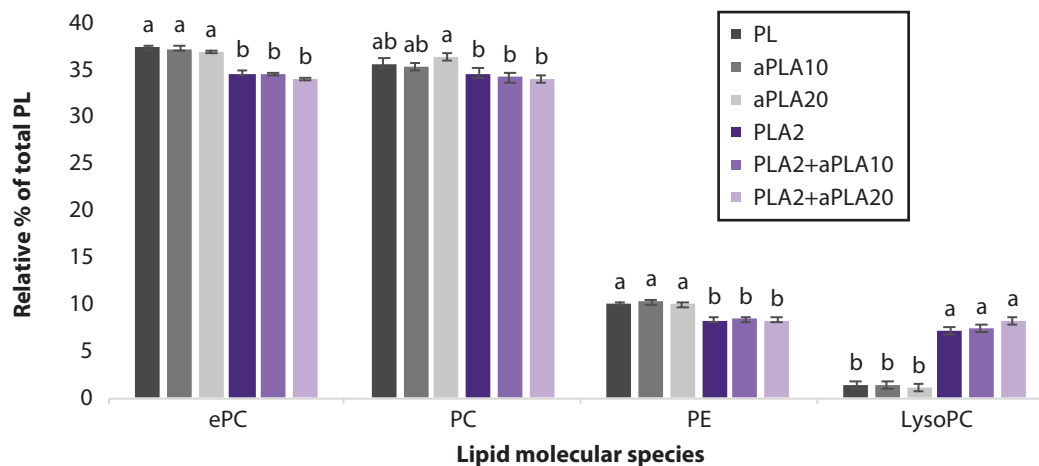


Figure 1. Effects of treatment on relative percent of ether-linked phosphatidylcholine (ePC), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and lysophosphatidylcholine (LysoPC) of total phospholipid (PL). ^{ab}Means within molecular species differ ($P < 0.01$). Treatments: PL (25 mg of PL); aPLA10 [PL + 25 μ g of phospholipase- A_2 antibody (aPLA2)]; aPLA20 (PL + 50 μ g of aPLA2); PLA2 (PL + 10 μ g of PLA2; 5) PLA2+aPLA10 (PL + PLA2 + 25 μ g of aPLA2); and PLA2+aPLA20 (PL + PLA2 + 50 μ g of aPLA2).

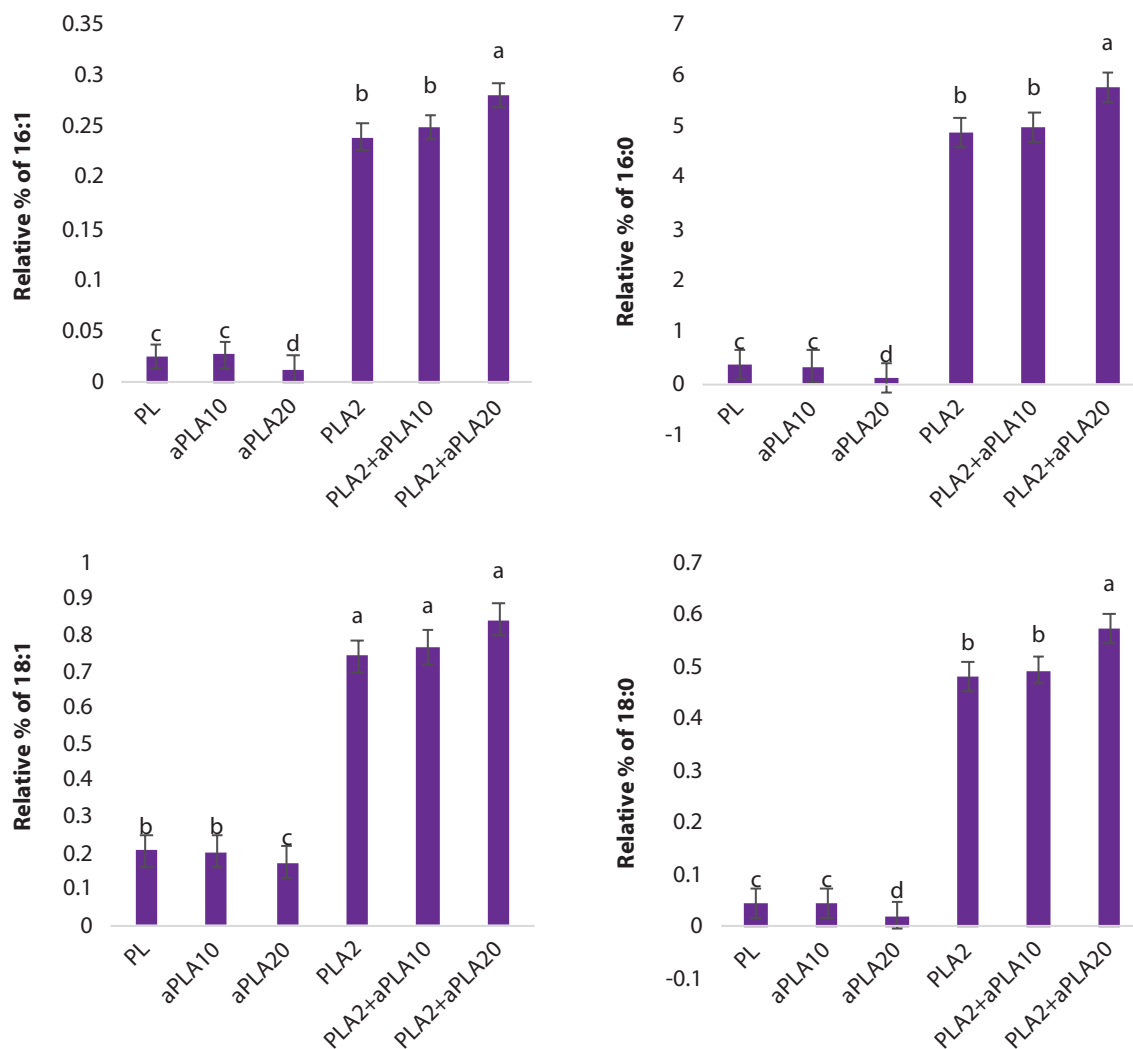


Figure 2. Effects of treatment on relative percent of individual lysophosphatidylcholine (LysoPC) species. ^{a-d}Means within treatment for each LysoPC species differ ($P < 0.05$). Treatments: PL (25 mg of PL); aPLA10 [PL + 25 μ g of phospholipase- A_2 antibody (aPLA2)]; aPLA20 (PL + 50 μ g of aPLA2); PLA2 (PL + 10 μ g of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25 μ g of aPLA2); and PLA2+aPLA20 (PL + PLA2 + 50 μ g of aPLA2).

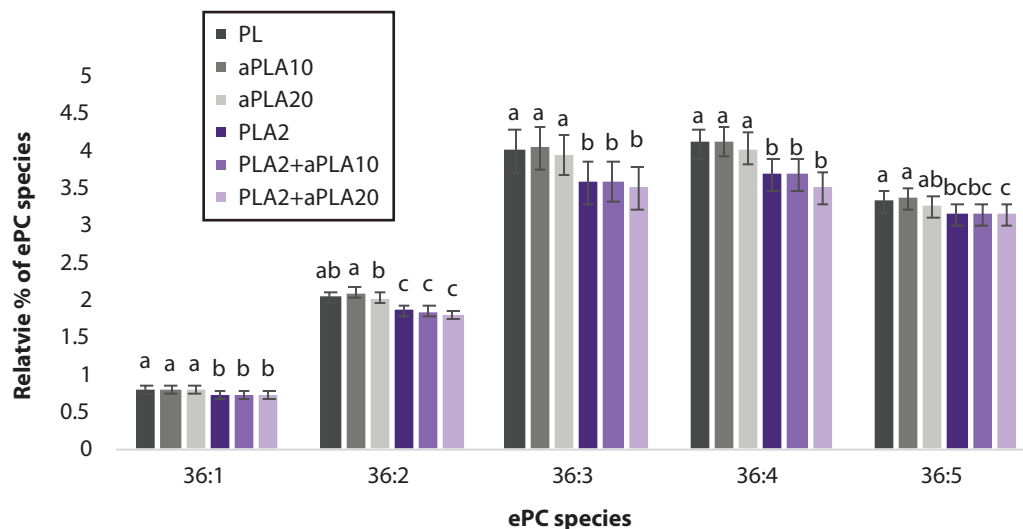


Figure 3. Effects of treatment on relative percent of ether-linked phosphatidylcholine (ePC) species. ^{a-c}Means within ePC species differ ($P < 0.05$). Treatments: PL (25 mg of PL); aPLA10 [PL + 25 μ g of phospholipase-A₂ antibody (aPLA2)]; aPLA20 (PL + 50 μ g of aPLA2); PLA2 (PL + 10 μ g of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25 μ g of aPLA2); and PLA2+aPLA20 (PL + PLA2 + 50 μ g of aPLA2).

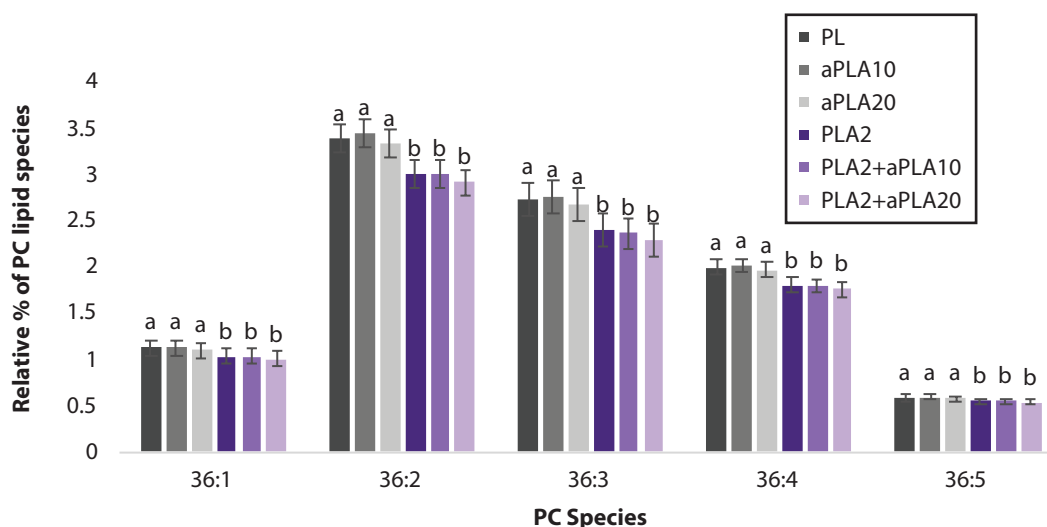


Figure 4. Effects of treatments on relative percent of phosphatidylcholine (PC) species. ^{ab}Means within PC species differ ($P < 0.05$). Treatments: PL (25 mg of PL); aPLA10 [PL + 25 μ g of phospholipase-A₂ antibody (aPLA2)]; aPLA20 (PL + 50 μ g of aPLA2); PLA2 (PL + 10 μ g of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25 μ g of aPLA); and PLA2+aPLA20 (PL + PLA2 + 50 μ g of aPLA2).

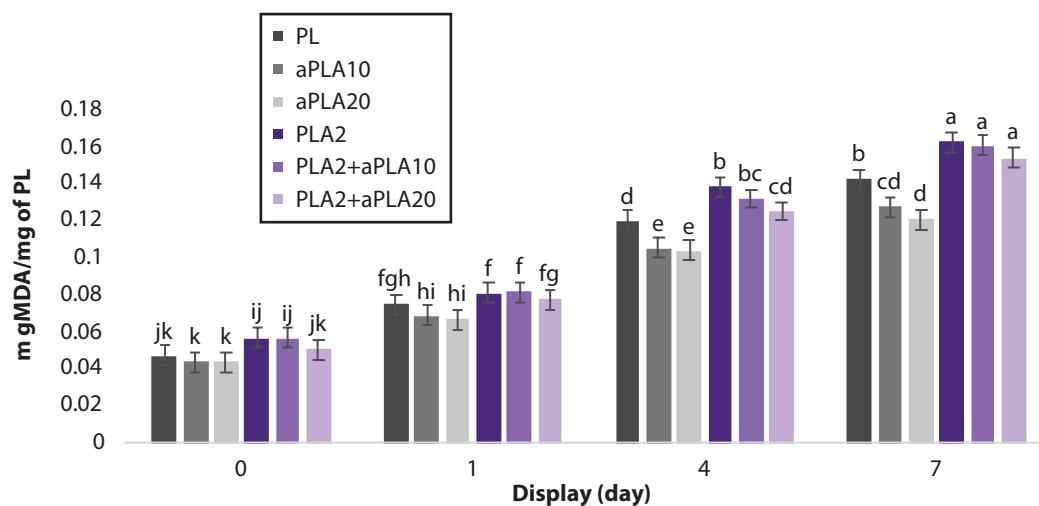


Figure 5. Effects of display and treatment on lipid oxidation analysis. ^{a-k}Means within bars differ ($P < 0.01$). MDA = malondialdehyde; PL = phospholipid. Treatments: PL (25 mg of PL); aPLA10 [PL + 25 μ g of phospholipase-A₂ antibody (aPLA2)]; aPLA20 (PL + 50 μ g of aPLA2); PLA2 (PL + 10 μ g of PLA2); 5) PLA2+aPLA10 (PL + PLA2 + 25 μ g of aPLA2); and PLA2+aPLA20 (PL + PLA2 + 50 μ g of aPLA2).