Published in final edited form as:
Chem Biol Interact. 2011 May 30; 191(1-3): 255–260. doi:10.1016/j.cbi.2011.01.032.

Catalytic reduction of carbonyl groups in oxidized PAPC by Kvβ2 (AKR6)

Zhengzhi Xie[a,1], **Oleg A. Barski**[a,1], **Jian Cai**[b], **Aruni Bhatnagar**[a], and **Srinivas M. Tipparaju**[a,*]

[a] Diabetes and Obesity Center, University of Louisville, Louisville, KY 40202, United States

[b] Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40202, United States

Abstract

The β-subunits of the voltage-gated potassium channel (Kvβ) belong to the aldo-keto reductase superfamily. The Kvβ-subunits dock with the pore-forming Kv α-subunits and impart or accelerate the rate of inactivation in Kv channels. Inactivation of Kv currents by Kvβ is differentially regulated by oxidized and reduced pyridine nucleotides. In mammals, AKR6 family is comprised of 3 different genes Kvβ1-3. We have shown previously that Kvβ2 catalyzes the reduction of a broad range of carbonyls including aromatic carbonyls, electrophilic aldehydes and prostaglandins. However, the endogenous substrates for Kvβ have not been identified. To determine whether products of lipid oxidation are substrates of Kvβs, we tested the enzymatic activity of Kvβ2 with oxidized phospholipids generated during the oxidation of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC). Electrospray ionization mass spectrometric analysis showed that Kvβ2 catalyzed the NADPH-dependent reduction of several products of oxPAPC, including 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine (POVPC), 1-palmitoyl-2-(epoxycyclopentenone)-*sn*-glycero-3-phosphorylcholine (PECPC), 1-palmitoyl-2-(5,6)- epoxyisoprostane E2-*sn*-glycero-3-phosphocholine (PEIPC). These results were validated using high resolution mass spectrometric analysis. Time course analysis revealed that the reduced products reached significant levels for ions at *m*/*z* 594/596 (POVPC/PHVPC), 810/812 (PECPC/2H-PECPC) and 828/830 (PEIPC/2H-PEIPC) in the oxPAPC + Kvβ2 mixture ($p < 0.01$). These results suggest that Kvβ could serve as a sensor of lipid oxidation via its catalytic activity and thereby alter Kv currents under conditions of oxidative stress.

Keywords

Kvβ; Catalysis; Oxidized phospholipids; Potassium channel; Mass spectrometry

1. Introduction

β-subunits of the voltage gated potassium channel belong to the aldo-keto reductase (AKR) superfamily 6 (AKR6). The function of voltage-gated potassium channel (Kv) is essential for several physiological processes, including muscle contraction, neuronal excitation, and secretion. Despite extensive investigation, the exact biological role of Kvβ-subunit remains

*Corresponding author at: Diabetes and Obesity Center, 580 South Preston Street #421B, University of Louisville, Louisville, KY 40202, United States. Tel.: +1 502 852 4221; fax: +1 502 852 3663. smtipp01@louisville.edu (S.M. Tipparaju).

[1]These authors made equal contribution.

Conflict of interest statement

None.

unclear. Identification of Kvβ substrates is important for understanding the biological role of Kv channels, how they are regulated, and how the activity of these channels could be therapeutically altered to treat diseases such as hypertension, epilepsy, and arrhythmias. Our previous studies have shown that Kvβ subunits bind to pyridine nucleotides with high affinity [1,2]. Sequence analysis of Kvβs shows that the proteins belong to the AKR superfamily [3], however, their catalytic properties and substrate specificities remain poorly understood. Recent work by Weng et al. shows that these proteins display weak catalytic activity with model chemical substrates such as 4-cyanobenzaldehyde and 4-carboxybenzaldehyde [4]. In addition, investigations in our laboratory have shown that Kvβ can reduce both aldehydes and ketones, and that naturally occurring compounds such as POVPC (1-palmitoyl-2-oxovaleroyl-3-phosphatidylcholine), and PGJ_2 have highest specific activity with Kvβ2 [5].

Products of lipid peroxidation, such as POVPC, however, are seldom generated in isolation. Lipid peroxidation generates several structurally similar aldehydes and ketones, several of which could also be potential Kvβ substrates. Hence, to identify which products of lipid peroxidation are reduced by Kvβ, we examined the activity of this protein with oxidized 1-palmitoyl-2-arachidonoyl-3-phosphotidyl choline (oxPAPC). The oxPAPC is a major component of minimally modified LDL (mmLDL) [6]. It has been detected in the atherosclerotic lesions of animals and humans [7,8]. Oxidation of PAPC generates several highly reactive carbonyl compounds. Structurally, a typical oxidation product of PAPC contains a glycerol backbone with a palmitoyl group esterified at the *sn1* position, an oxidized fatty acyl group at the *sn2* position, and a phosphatidyl choline head group at the *sn3* position (Fig. 1A). In a previous study, we have shown that aldose reductase (AKR1B1), and other members of AKR superfamily have high catalytic activity for products of oxPAPC that contain aldehyde groups [9].

To determine which of the oxidation products of PAPC are reduced by Kvβ, we preformed electrospray ionization mass spectrometric (ESI-MS) analysis of oxPAPC incubated with Kvβ2. Our results show that Kvβ2 efficiently reduces both aldehydes and ketones in oxidized phospholipids and therefore might play a regulatory role in sensing the carbonyl products that are generated during lipid oxidation.

2. Materials and methods

2.1. Kvβ2 expression and purification

The C-terminal domain of rat Kvβ2 (amino acids 39–367) with a His tag at its N-terminus was expressed in the BL-21 strain of *Escherichia coli*, as described earlier [1]. The His-tagged protein was purified using Profinia Protein Purification System (Bio-Rad) with a 1 ml Bio-Scale Mini Profinity IMAC cartridge (Bio-Rad) according to manufacturer's instructions. The purified protein was suspended in 0.2 M potassium phosphate buffer (pH 7.4) immediately after the purification using a 10 ml Bio-Gel P-6 Desalting cartridge (Bio-Rad). The concentration of the purified protein was measured using Bradford's assay [10].

2.2. Preparation of air oxidized PAPC (oxPAPC)

Air oxidation of synthetic 1-palmitoyl-2-arachidonoyl-3-phosphotidyl choline (PAPC) was preformed as described before [9]. Briefly, 100 μg of PAPC in chloroform (Avanti Polar Lipids) was dried under N_2 on the bottom of a 4 ml glass vial (National Scientific). The dry lipid was exposed to air for 72 h in dark at room temperature. The extent of the oxidation was determined using ESI-MS. The oxidation product was stored in −80 °C until use.

2.3. Reduction of oxPAPC using Kvβ2 and NADPH

Reduction of carbonyl groups of lipid oxidation products in oxPAPC to their corresponding alcohols by Kvβ2 was followed by using ESI-MS analysis as described earlier [5] with some modifications. The 250 μl reaction mixtures containing purified Kvβ2 protein (20 μM), NADPH (150 μM), and oxPAPC (88 μg/ml) in 0.2 M potassium phosphate buffer (pH7.4), were incubated in a Teflon tube (Upchurch Scientific) at 37 °C. Additional NADPH was added to the reaction mixture after 3 h, 6 h, and 12 h of incubation, and additional Kvβ2 was added into the reaction mixture after 6 h of incubation. The reaction was stopped by freezing the reaction mixture at −80 °C at 3 h, 6 h, 12 h, or 22 h, respectively. The samples were stored at −80 °C until extraction and ESI-MS analysis.

2.4. Analysis of the reduced product using ESI-MS

Identification and quantification of carbonyl substrates and alcohol products were performed as described earlier with minor modification [5,9]. The reduced product was extracted using the Bligh and Dryer procedure [11]. The chloroform extract was dried under N_2, and dissolved in 10 mM NH_4OAc in methanol–chloroform (2:1, v/v). The solution was injected into a MicroMass ZMD 2000 mass spectrophotometer (Waters-Micromass, Milford, MA) using a Harvard syringe pump (Harvard Apparatus) at a flow rate of 10 μl per min. Positive ion spectra were acquired over the mass range of 20–1000 atomic mass with following operating parameters: capillary voltage 4.34 kV, cone voltage 35 V, extractor voltage 9 V, RF lens voltage 0.9 V, source block 100 °C, desolvation temperatures 200 °C.

2.5. High resolution mass spectrometry analysis

After extraction, the chloroform extract was dried under N_2, and dissolved in 7.5 mM NH_4OAc in methanol. High resolution mass spectrometry analysis was performed on a LTQ Orbitrap XL Mass Spectrometer (Thermo) equipped with a TriVersa NanoMate ion source (Advion BioSciences) and an electrospray chip (nozzle inner diameter 5.5 μm). TriVersa NanoMate was operated in positive mode at gas pressure 0.05 psi and ionization voltage of 1.2 kV. MS scans were acquired in positive ion mode with a mass range from 100 to 1500 and resolution at 100,000.

2.6. Data analysis

High-accuracy *m*/*z* values were obtained from LTQ-Orbitrap spectra averaged over 5 min using Qual Browser Xcalibur 2.0. Peak intensity values were acquired from ESI-MS spectra averaged over 3.5 min using Masslynx 3.2. Formation of alcohol products were calculated based on the intensities of [M] and [M + 2] peaks and reported as a percent conversion. One-way ANOVA analyses were performed using SigmaStat 3, and time course figures were prepared using Sigmaplot 10. Data was collected from 3 or more experiments for each time point.

3. Results

3.1. Air oxidation of PAPC and generation of lipid-carbonyls

As shown in Fig. 1, air oxidation of PAPC for 72 h resulted in the formation of oxPAPC containing several compounds with higher and lower *m*/*z* values than the parent compound (*m*/*z* 782). As previously reported, the major ion at *m*/*z* 594 was ascribed to POVPC (1-palmitoyl-2-oxo-valaroyl-3-phosphotidyl choline) *compound 1* [9], whereas the ions at *m*/*z* 810 and 828 were epoxy isoprostane *compounds 2 and 3*: 1-palmitoyl-2-(5,6)-epoxyisoprostane E2-*sn*-glycero-3-phosphocholine (PEIPC) and 1-palmitoyl-2-(epoxycyclopentenone)-*sn*-glycero-3-phosphorylcholine (PECPC) respectively (Fig. 1A).

All the three molecules contain reactive carbonyl groups and POVPC has been previously reported to be reduced by aldose reductase [9].

3.2. Reduction of carbonyl groups in oxPAPC mixture

To identify which of the carbonyl groups generated in oxPAPC are reduced by Kvβ, and to determine the substrate preference of Kvβ2, we incubated oxPAPC with recombinant Kvβ2. Because enzymatic reduction leads to the formation of alcohols from aldehydes or ketones, we expected that the reduction products generated in the reaction mixture would show an increase in their *m*/*z* value by 2 atomic mass units over the un-reduced substrates. Hence, we monitored the ratio between intensities of [M] and [M + 2] peaks to identify reduction products.

Incubation with Kvβ2 resulted in the appearance of several new phospholipid species (Fig. 2A). For example, we found an increase in the ratio of 596.3 (1-palmitoyl-2-hydroxyvaleroyl phosphatidylcholine, PHVPC) to 594.3 (POVPC), Fig. 2A and B. This is consistent with our previous report that NADPH was consumed in presence of Kvβ2 and purified reagent POVPC [5]. Similarly, two dalton increments were observed for ions with *m*/*z* 812 and 830 which indicated the formation of 2H-PEIPC and 2H-PECPC derived from reduction of PEIPC and PECPC, respectively (Fig. 2C and D). These data clearly demonstrate that Kvβ2 converts aldehydes and ketones in the oxPAPC to their corresponding alcohols, and that Kvβ2 has a broad substrate activity required to reduce several oxidized phospholipids containing carbonyl groups.

3.3. High resolution mass spectrometry analysis

The relative peak intensity of [M + 2] was used to monitor the progress of reduction. However, the ESI-MS analysis cannot distinguish the [M + 2] peak which arises from reduction (e.g. M + 2H) or the isotopic peak (e.g. M with 2 ^{13}C) which naturally occurs even if the reduction does not occur. Fortunately, oxPAPC compounds only contain C, H, O, and N, and their [M + 2] isotopic peaks are relatively small. So the lack of mass resolution of ESI-MS analysis is not a serious problem if a large amount of the reduction products is present. However, at the beginning of the reaction, when the amount of reduction products is small, the contribution of isotopic peak to [M + 2] cannot be ignored. Therefore, we used high resolution MS analysis to overcome this problem.

For high resolution mass analysis, oxPAPC was incubated with Kvβ2 for 0, 3, and 6 h. After extraction, the reaction products were applied on LTQ-Orbitrap MS for high resolution mass spectrometry analysis. As shown in Fig. 3, when enzyme was absent, there was no PHVPC peak (theoretical *m*/*z*: 596.39220). Instead, a peak with *m*/*z* at 596.38256 was found, which was identified as POVPC ^{13}C isotopic peak (theoretical *m*/*z*: 596.38325). This peak did not increase with Kvβ2 incubation at 3 and 6 h. In contrast to this, the PHVPC peak (observed *m*/*z* 596.39139) was seen to be progressively increased upon incubation with Kvβ2 at 3 and 6 h. This result clearly validates the ESI-MS (Fig. 2) data, and demonstrates the reduction of the aldehydes (POVPC) to an alcohol (PHVPC).

3.4. Time course effects on product formation

To quantify the product formation over the time of incubation, we monitored the major ion pairs at *m*/*z* 594/596, 810/812 and 828/830 at different time points of reaction. As shown in Fig. 4A, a progressive increase in the conversion of POVPC (594 *m*/*z*) to PHVPC (596 *m*/*z*) was observed. Indeed, the conversion could be detected as early as 3 h. At 6 h, the differences between the reaction mixture and no enzyme control were highly significant ($p < 0.01$). Similar results were observed for the ion at *m*/*z* 828, which represents the reduction of PIEPC to 2H-PIEPC (*m*/*z* 830). The reaction mixture containing enzyme was significantly

different at the 6 h and 22 h time point compared with control (no enzyme). The relative increase in the ion intensity at *m*/*z* 821, which indicates the conversion of PECPC to its corresponding alcohol 2H-PECPC, was also found to be higher in the sample incubated with the enzyme than in the absence of the enzyme. However, the reaction had a delayed start because the overall conversion did not alter even at 6 h time point. For this reaction, the conversion was highest at the 22 h time point and was statistically significant compared with the no-enzyme control group. These data demonstrate that the carbonyl groups in the oxPAPC mixture are catalytically reduced to their corresponding alcohols by Kvβ2, and that POVPC and PEIPC are preferred over PECPC.

4. Discussion

This study demonstrates that Kvβ2 catalyzes the reduction of several structurally similar compounds in oxPAPC mixture, which suggests that Kvβ2 subunits can play a regulatory role in sensing the carbonyl products that are generated during lipid oxidation. Sequence homology studies indicate that Kvβ subunits belong to the AKR superfamily of proteins. The β-subunits of shaker potassium channel have β/α_8 barrel structure with tight NADPH binding and have 20–25% homology to other AKRs [12]. Kvβ 1 and 3 consist of the long N-terminus which acts as a ball and inactivates the channel by plugging the pore of the Kv channel, whereas in Kvβ2 the N-terminus is absent and therefore it can only accelerate the self inactivating Kv channels [13].

The Kvβ subunits bind to the cytosolic domain of Kv channels and modulate their gating properties. We have shown previously that pyridine nucleotides can differentially regulate the Kv channel inactivation in which oxidized pyridine nucleotides $NAD(P)^+$ abolish inactivation and reduced pyridine nucleotides NAD(P)H restore inactivation [2,14]. We have also examined the catalytic activity of Kvβ2 and have identified that the protein displays higher activity with naturally occurring compounds, such as PGJ_2 and POVPC, whereas modest reduction was monitored with 12-oxo-ETE and steroid containing compounds.

In comparison with other AKRs such as aldose reductase (AKR1B), Kvβ2 is a much slower enzyme. In our previous publications we reported catalytic activity for Kvβ2 with k_{cat} for POVPC and PGJ_2 of 0.078 and 0.088 min^{-1}, respectively [5], whereas k_{cat} of aldose reductase with POVPC is 36.2 min^{-1} [9]. Based on these measurements using POVPC as substrate, aldose reductase is estimated to be 464 times more active than Kvβ2. Therefore, it is likely that Kvβ2 is not a detoxification enzyme like some other AKRs. However, the unique property of Kvβ subunits is that it is the only known AKR that binds to ion channels (Kv channels) and modulates channel gating and trafficking [15]. Therefore, the catalytic activity of Kvβ2 with the products of lipid oxidation may serve as a sensor of oxidative changes in the cell membrane, which will allow the regulation of potassium currents in response to the metabolic/redox status of the cell.

Our findings are in agreement with previous reports, which show that oxidation of PAPC results in generation of several classes of carbonyl compounds [6]. Among them, one of the most reactive phospholipid aldehyde generated is POVPC and it has been suggested that the generation of POVPC within the vessel wall could contribute to atherosclerotic lesion formation and progression [7,16,17]. In general, oxidized phospholipids have been implicated in the pathogenesis of several chronic inflammatory diseases such as arthritis, inflammatory bowel disease, and multiple sclerosis. Nevertheless, the ability to modulate ion channels or their subsidiary sub-units has not been examined.

The present study shows that Kvβ2 reduces several carbonyl products generated from the oxidation of membrane lipids. We observed significant catalytic activity of Kvβ with POVPC, PEIPC, as well as PECPC. These findings attest to the possibility that Kvβ2 subunit can sense metabolic changes in the cell and play a regulatory role in ion channel modulation. Although we used Kvβ2 for our studies, because of high sequence homology among different Kvβ subunits, it is likely that other Kvβ subunits, i.e., Kvβ1 and Kvβ3, can also catalyze the reduction of oxidized phospholipids, however, future experiments are required to determine the catalytic properties of other β-subunits and how they are different from Kvβ2 [2,12]. Further work is also required to understand how individual species generated within oxidized lipids regulate electrical activity by regulating Kvβ catalysis and thereby the inactivation of Kv currents.

In summary, our studies show that several oxidized phospholipid generated by the oxidation of PAPC are reduced by Kvβ2. These results were validated using high resolution mass spectrometry. We report that major carbonyl compounds with *m*/*z* 594, 810 and 828 are reduced by Kvβ2. High resolution mass spectrometry evidence was used to validate the reduction of POVPC at different time points, and the time-course of the reduction for the three major species was monitored to demonstrate the reduction process in oxPAPC mixture. Taken together, these data demonstrate that Kvβ2 catalyzes the reduction of several oxidized phospholipids. Based on these findings, we speculate that oxidized phospholipid generated during oxidative stress might be able to decrease the inactivation of Kv currents by oxidizing NADPH bound to Kvβ-subunit and that changes in Kv conductance via Kvβ may be one mechanism by which cells respond to oxidative stress.

Acknowledgments

The authors thankfully acknowledge the research support provided by NIH (HL-54477, HL-59378, ES-11860; to AB), NCRR (RR024489), University of Louisville Clinical and Translational Science award 20017 and HL-089372 (to OAB), and American Heart Association Beginning Grant-in-Aid 0865466D (to SMT).

References

1. Liu SQ, Jin H, Zacarias A, Srivastava S, Bhatnagar A. Binding of pyridine nucleotide coenzymes to the beta-subunit of the voltage-sensitive K+ channel. J Biol Chem. 2001; 276:11812–11820. [PubMed: 11278398]
2. Tipparaju SM, Liu SQ, Barski OA, Bhatnagar A. NADPH binding to beta-subunit regulates inactivation of voltage-gated K(+) channels. Biochem Biophys Res Commun. 2007; 359:269–276. [PubMed: 17540341]
3. McCormack T, McCormack K. Shaker K+ channel beta subunits belong to an NAD(P)H-dependent oxidoreductase superfamily. Cell. 1994; 79:1133–1135. [PubMed: 8001150]
4. Weng J, Cao Y, Moss N, Zhou M. Modulation of voltage-dependent Shaker family potassium channels by an aldo-keto reductase. J Biol Chem. 2006; 281:15194–15200. [PubMed: 16569641]
5. Tipparaju SM, Barski OA, Srivastava S, Bhatnagar A. Catalytic mechanism and substrate specificity of the beta-subunit of the voltage-gated potassium channel. Biochemistry. 2008; 47:8840–8854. [PubMed: 18672894]
6. Subbanagounder G, Watson AD, Berliner JA. Bioactive products of phospholipid oxidation: isolation, identification, measurement and activities. Free Radic Biol Med. 2000; 28:1751–1761. [PubMed: 10946217]
7. Subbanagounder G, Leitinger N, Schwenke DC, Wong JW, Lee H, Rizza C, Watson AD, Faull KF, Fogelman AM, Berliner JA. Determinants of bioactivity of oxidized phospholipids. Specific oxidized fatty acyl groups at the sn-2 position. Arterioscler Thromb Vasc Biol. 2000; 20:2248–2254. [PubMed: 11031211]
8. Watson AD, Leitinger N, Navab M, Faull KF, Horkko S, Witztum JL, Palinski W, Schwenke D, Salomon RG, Sha W, Subbanagounder G, Fogelman AM, Berliner JA. Structural identification by

mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. J Biol Chem. 1997; 272:13597–13607. [PubMed: 9153208]

9. Srivastava S, Spite M, Trent JO, West MB, Ahmed Y, Bhatnagar A. Aldose reductase-catalyzed reduction of aldehyde phospholipids. J Biol Chem. 2004; 279:53395–53406. [PubMed: 15465833]
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem. 1976; 72:248–254. [PubMed: 942051]
11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959; 37:911–917. [PubMed: 13671378]
12. Barski OA, Tipparaju SM, Bhatnagar A. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. Drug Metab Rev. 2008; 40:553–624. [PubMed: 18949601]
13. Peri R, Wible BA, Brown AM. Mutations in the Kv beta 2 binding site for NADPH and their effects on Kv1.4. J Biol Chem. 2001; 276:738–741. [PubMed: 11024060]
14. Tipparaju SM, Saxena N, Liu SQ, Kumar R, Bhatnagar A. Differential regulation of voltage-gated K+ channels by oxidized and reduced pyridine nucleotide coenzymes. Am J Physiol Cell Physiol. 2005; 288:C366–C376. [PubMed: 15469953]
15. Pongs O, Schwarz JR. Ancillary subunits associated with voltage-dependent K+ channels. Physiol Rev. 2010; 90:755–796. [PubMed: 20393197]
16. Hill BG, Haberzettl P, Ahmed Y, Srivastava S, Bhatnagar A. Unsaturated lipid peroxidation-derived aldehydes activate autophagy in vascular smooth-muscle cells. Biochem J. 2008; 410:525–534. [PubMed: 18052926]
17. Srivastava S, Liu SQ, Conklin DJ, Zacarias A, Srivastava SK, Bhatnagar A. Involvement of aldose reductase in the metabolism of atherogenic aldehydes. Chem Biol Interact. 2001; 130–132:563–571.

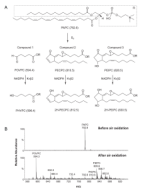

Fig. 1.

Structural representation of air oxidation products of PAPC and its reduction by Kvβ2. (A) The chemical structure of the parent compound PAPC is depicted with the non-substitutable group R represented in dotted box. Air oxidation of PAPC leads to the formation of reactive carbonyl groups at the *sn2* position, and reduction of these carbonyl groups by Kvβ2 in the presence of NADPH leads to the formation of corresponding alcohols. *Compound 1* (POVPC), *sn2* group: oxo-valeroyl group can be reduced by Kvβ2 to form PHVPC (596.4 *m*/*z*). *Compound 2* (PECPC), *sn2* group: epoxy-isoprostaonic group, can be reduced to 2H-PECPC (812.5 *m*/*z*) by Kvβ2. *Compound 3* (PEIPC), *sn2* group: hydroxyl-epoxyisoprostanoic, can be reduced by Kvβ2 to its corresponding 2H-PEIPC (830.5 *m*/*z*). (B) ESI-MS spectra of PAPC before air oxidation shows a well resolved positive ion at *m*/*z* 782.6 as indicated, which was air oxidized for 72 h to generate carbonyl containing compounds: POVPC (*m*/*z* 594.3), PECPC (*m*/*z* 810.6), and PEIPC (*m*/*z* 828.6).

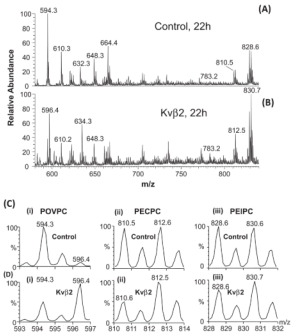

Fig. 2.

Kvβ2 catalyzes the reduction of aldehydes generated from air oxidation of PAPC. Aliquots of oxidized PAPC were incubated without (A) or with Kvβ2 (B) in potassium phosphate buffer (see Section 2). After the incubation, the phospholipids were extracted in chloroform/methanol/water and injected in to the electrospray. The oxidized compounds POVPC, PEIPC and PECPC remain unaltered in the absence of Kvβ2. Incubation with Kvβ2 led to an increase in the intensity at *m*/*z* 596.3, 812.5, 830.5, which represents the formation of the reduction products of POVPC, PECPC and PEIPC. Panels C and D, represent narrow-range spectra of the three products without (control; i–iii) or with Kvβ2 (i–iii) respectively.

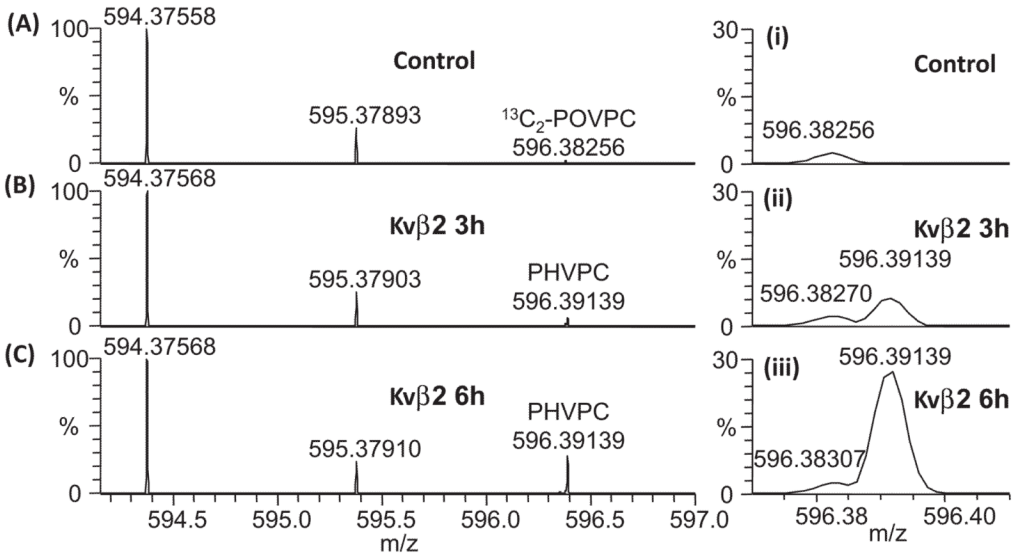

Fig. 3.

Identification of products by high resolution mass spectrometry. Aliquots of reaction mixture containing oxPAPC without or with Kvβ2 were analyzed by Orbitrap high resolution mass spectrometer at 3 and 6 h time points. (A) In control group without Kvβ2, no PHVPC product at *m*/*z* 596.38 was identified. (B) After 3 or (C) 6 h of incubation with Kvβ2 a progressive increase in the intensity at *m*/*z* 596.39139 was noted. Corresponding insets i, ii and iii (narrow-range spectra) representing the formation of PHVPC (*m*/*z* 596.39) at 0, 3 and 6 h time points respectively.

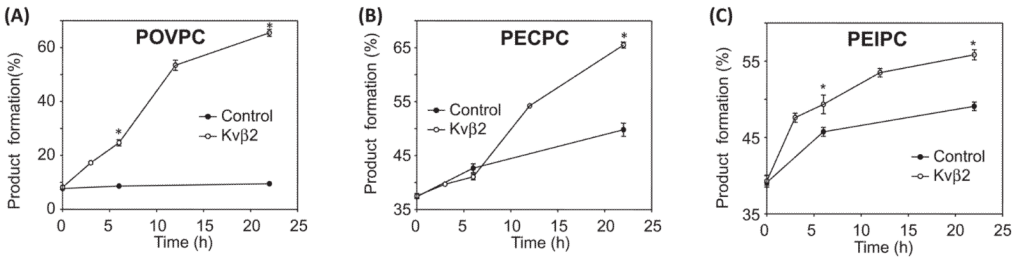

Fig. 4.

Time course of carbonyl reduction by Kvβ2 Reaction mixtures containing Kvβ2 and oxPAPC were incubated and monitored for the generation of reduced products at different time points without or with Kvβ2. The reaction was stopped at 0, 3, 6, 12, and 22 h by freezing at −80 °C. Phospholipids were extracted by chloroform/methanol/water mixture and analyzed by electrospray. The formation of reduced products is plotted as percentage conversion. Panel (A) shows the time course of PHVPC (*m*/*z* 596) product formation compared with control. Panel (B) and (C) shows the 2H-PECPC (*m*/*z* 812) and 2H-PEIPC (*m*/*z* 830) product respectively. $n = 3$ separate reactions, $^{*}p < 0.01$.