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Lys 43 and Asp 46 in α-helix 3 of uteroglobin are essential for its phospholipase A_2-inhibitory activity☆

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Abstract

Uteroglobin (UG) is an anti-inflammatory, secreted protein with soluble phospholipase A_2 ($sPLA_2$)-inhibitory activity. However, the mechanism by which UG inhibits $sPLA_2$ activity is unknown. UG is a homodimer in which each of the 70-amino acid subunits forms four α-helices. We previously reported that $sPLA_2$-inhibitory activity of UG may reside in a segment of α-helix 3 that is exposed to the solvent. In addition, it has been suggested that UG may inhibit $sPLA_2$ activity by binding and sequestering Ca^{++}, essential for $sPLA_2$ activation. By site-specific mutation, we demonstrate here that Lys 43 Glu, Asp 46 Lys or a combination of the two mutations in the full-length, recombinant human UG (rhUG) abrogates its $sPLA_2$-inhibitory activity. We demonstrate further that recombinant UG does not bind Ca^{++} although when it is expressed with histidine-tag (H-tag) it is capable of binding Ca^{++}. Taken together our results show that: (i) Lys 43 and Asp 46 in rhUG are critical residues for the $sPLA_2$-inhibitory activity of UG and (ii) Ca^{++}-sequestration by rhUG is not likely to be one of the mechanisms responsible for its $sPLA_2$-inhibitory activity. © 2002 Elsevier Science (USA). All rights reserved.

Steroid hormones regulate the expression of many proteins; however, the physiological functions of only a limited number have been clearly identified. Blastokinin [1] or uteroglobin (UG; [2]) is a steroid-inducible, multifunctional, secreted protein with potent anti-inflammatory/immunomodulatory properties [3]. Although it was first discovered in the rabbit uterus, this protein is expressed in many extrauterine tissues, including the thymus, pituitary gland, lungs, gastrointestinal tract, pancreas, mammary gland, prostate, and the seminal vesicle [4]. UG is also present in the blood [5,6] and in urine [7], although it is not synthesized in the kidneys. Currently, this protein is known by several names, which are primarily derived from the organ or body fluid in which it is detectable or from the type of xenobiotics with which it interacts. Thus, it is called the progesterone-binding protein [8], Clara cell 10 kDa protein [9], urine protein-1 [7], polychlorinated biphenyl-binding protein [10], and retinol-binding protein [11]. Recently, the UG/Clara cell family of proteins has been classified as members of a new superfamily called "secretoglobins" [12].

Structurally, UG is a homodimer in which the two 70-amino acid subunits are covalently linked in an antiparallel orientation by two inter-chain disulfide bonds [3]. Each monomer consists of four α-helices and there is a β-turn between α-helix-2 and -3. The UG gene, in all mammals including humans, consists of three exons and two introns. Its 5′ flanking region contains several steroid hormone-response elements, which regulate the tissue-specific expression of the UG gene [13–15]. Moreover, a polypeptide hormone, prolactin, appears to enhance further steroid-induced UG gene-expression

☆ *Abbreviations:* rhUG, recombinant human uteroglobin; $sPLA_2$, soluble phospholipase A_2; DTNB, 5,5′-dithiobis-nitrobenzoate; IFNγ, interferon-γ; H-tag, histidine-tag; AA, arachidonic acid; NMR, nuclear magnetic resonance.

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[16–19]. Recently, a pro-inflammatory cytokine, interferon-γ (IFN-γ), was found to have a stimulatory effect on UG production in the murine lungs [20] and an IFNγ-response element in the 5′-promoter region of the mouse UG gene has been identified [21]. These results suggest a potential role of this protein in the regulation of immunological/inflammatory processes.

The production of rabbit [22] and human [23] recombinant UGs in *Escherichia coli* allowed the comparison of their structural features as determined by X-ray crystallography [24,25] and by multidimensional NMR [26]. The results of these and other studies [27,28] showed that rabbit and human UGs are indistinguishable proteins both structurally [29] and functionally [23].

Several years ago, we demonstrated, for the first time, that rabbit UG inhibits the secretory phospholipase A_2 ($sPLA_2$; E.C.3.1.1.4) activity [30]. We also reported that both natural and recombinant rabbit and human UGs are potent inhibitors of $sPLA_2$ activity [22,23]. Subsequently, we [31–33] and others [34–36] have demonstrated that the $sPLA_2$-inhibitory and anti-inflammatory activities of UG may reside in a nonapeptide region (residues 39–47) of the α-helix-3 of UG. Furthermore, the alteration of Lys 43 or Asp 46 in this nonapeptide abrogated its $sPLA_2$-inhibitory activity [31].It is well known that the biochemical properties of a synthetic oligopeptide derived from a protein may not necessarily reflect those of the corresponding region within the intact protein. Thus, it is important to determine whether mutation of Lys 43 and/or Asp 46 within the intact hUG protein would alter its $sPLA_2$-inhibitory activity. The molecular mechanism of the inhibition of $sPLA_2$ activity by UG and by its derived peptides remains unclear. Recently, it has been demonstrated that histidine-tag (H-tag) recombinant hUG (rhUG) inhibits soluble PLA_2 ($sPLA_2$) activity and proposed that this inhibition of sPLA2 by hUG is due to its ability to bind and sequester Ca^{++} [37], essential for $sPLA_2$ activation. However, in these experiments the H-tag was not cleaved off before its use in $sPLA_2$ inhibition assay, raising the possibility that the presence of the H-tag in rhUG may have caused Ca^{++}-binding and sequestration.

Therefore, the present investigations were conducted to determine whether: (a) mutation of Lys 43 or Asp 46 in α-helix-3 of full-length rhUG alters its $sPLA_2$-inhibitory activity and (b) rhUG, both with and without the H-tag bind $^{45}Ca^{++}$. Our results show that: (i) mutation of either Lys 43 to Glu and/or Asp 46 to Lys abrogates the $sPLA_2$-inhibitory activity of rhUG and (ii) while the H-tag-rhUG avidly binds $^{45}Ca^{++}$, rhUG without H-tag show no binding, although the $sPLA_2$-inhibitory activities of both H-tag and non-H-tag proteins remain unaltered. Taken together, these results suggest that: (a) Lys 43 and Asp 46 in hUG are crucial for the $sPLA_2$-inhibitory activity of hUG and (b) Ca^{++}-binding by recombinant hUG is a function of H-tag associated with the mature rhUG and this property cannot be demonstrated by rhUG without the H-tag. Thus, Ca^{++}-sequestration does not appear to be one of the mechanisms by which hUG inhibits the $sPLA_2$ activity.

Materials and methods

Reagents and chemicals. Porcine pancreatic secretory phospholipase A_2 ($sPLA_2$ IB) and arachidonic acid (AA) were purchased from Boehringer. [^{14}C]Phosphatidylcholine and [^{14}C]AA were from Amersham Pharmacia Biotech. Prechanneled Silica Gel (250 μm) thin layer chromatography (TLC) plates were from Analtech. All other analytical-grade reagents were from Sigma (St. Louis, MO), Fisher (Pittsburgh, PA) or Amersham (Piscataway, NJ).

Site-specific mutagenesis of hUG. Site-specific mutagenesis of hUG was carried out by replacing a *Bcl*I–*Hin*dIII fragment from pGEL101 [23] with synthetic, double-stranded oligonucleotides carrying codon substitutions at amino acid positions 43 and/or 46. Briefly, pGEL101 was grown in *dam*/*dcm E. coli* JM110 [38], purified, and digested by *Bcl*I and *Hin*dIII. This excised the nucleotides encoding amino acid residues 40–70 and the 3′ non-translated region of the hUG cDNA. The excised region was replaced with phosphorylated, synthetic DNA fragments of the following sequences (mutant codons are underlined):

TGATCAAGACATGAGGGAGGCAGGGGCTCAGCTGAAG
GAACTGGTGGACACCCTCCCCCAAAAGCCCAGAGAAACA
TCATTAAGCTCATGGAAAAAATAGCCCAAAGCTCACTGTG
TAAAAGCTT

TGATCAAGACATGAGGGAGGCAGGGGCTCAGCTGAAG
AAGCTGGTGAAAACCCTCCCCCAAAAGCCCAGAGAAAGC
ATCATTAAGCTCATGGAAAAAATAGCCCAAAGCTCACTGT
GTAAAAGCTT

TGATCAAGACATGAGGGAGGCAGGGGCTCAGCTGAAG
GAACTGGTGAAAACCCTCCCCCAAAAGCCCAGAGAAAGC
ATCATTAAGCTCATGGAAAAAATAGCCCAAAGCTCACTGT
GTAAAAGCTT

The designations of the three constructs containing specific mutation(s) are: pGEL101-MH_1 (Lys 43 Glu), pGEL101-MH_2 (Asp 46 Lys), and pGEL101-MH_3 (Lys 43 Glu and Asp 46 Lys). After the constructs were sequenced and the specific mutations were confirmed, they were electroporated into BL21 (DE3). Mutant proteins were purified as described for the wild-type recombinant human UG [23] with minor modifications. Expression levels and yields of mutant proteins after purification were essentially identical to those previously described for the wild-type rhUG [23]. Briefly, cell lysates of the wild-type and mutated strains were prepared by the use of B-Per^{II} reagent (Pierce Chemical). The lysate was centrifuged twice for 1 h each at 20,000*g* to remove cellular debris. Chromatographic purification of the wild-type and mutated proteins was achieved by Sephacryl S-200 superfine columns (Pharmacia, 2.5 × 100 cm), cation-exchange columns (Econopak S, Bio-Rad), and Sephadex G-50 (superfine) columns (Pharmacia, 1.5 × 70 cm), respectively. Fractions containing purified proteins were pooled and lyophilized in small aliquots (2 ml each). The protein content was determined by the Bradford method [39], using a kit (Bio-Rad) according to manufacturer's specifications. SDS–PAGE and silver staining determined the homogeneity of the wild-type and mutant proteins. Purified MH_1, MH_2, and MH_3 proteins were dimeric and did not contain reduced Cys residues as determined by DTNB (5,5′-dithiobis-nitrobenzoate, not shown) reaction, and were dimeric like the wild-type rhUG.

Expression and purification of H-tag rhUG. To express H-tag rhUG, an antisense primer containing four histidine codons immediately preceding the stop codons was used to amplify the hUG coding sequence by PCR using pGEL-101 [23] as the template. The primer

oligonucleotides were designed to introduce *Nde*I and *Hin*dIII restriction sites so that restriction digest with these enzymes would produce a fragment containing both the initiation and termination codons of the hUG cDNA. The product of the PCR was precipitated with ammonium acetate and ethanol, digested with *Nde*I and *Hin*dIII, purified by agarose gel electrophoresis, and subcloned into an *E. coli* expression plasmid, containing both *lac* and *T7* promoters [40].

The H-tag rhUG was purified by immobilized metal affinity chromatography [41] on chelating Sepharose charged with Ni^{++} ion. Fractions containing the recombinant protein were pooled, dialyzed in PBS (pH 7.2), concentrated by treatment with Aquacide II, and applied to a HiLoad 16/60 Superdex 75 FPLC column. Trace-metal ions were removed from this H-tag protein by treatment with Chelex 100 (1 g of resin/2 mg of H-tag rhUG).

Binding of $^{45}Ca^{++}$ by H-tag rhUG and rhUG without H-tag. Calcium-45 ($^{45}Ca^{++}$)-binding by H-tag and non-H-tag hUG was performed by the use of calcium overlay technique and subsequent autoradiography according to a previously published method [42]. A sample each of H-tag- and non-H-tag hUG was resolved by SDS–PAGE [43] underdenaturing and reducing conditions. The separated protein bands were electrotransferred to nitrocellulose membranes [44] with a pore size of 0.1 μm using a solution containing 20% methanol, 0.025 M Tris, and 0.129 M glycine (pH 8.5) as the electrode buffer. After transfer, the membranes were soaked in a solution containing 60 mM KCl, 5 mM $MgCl_2$, and 10 mM imidazole–HCl (pH 6.8), and the membranes were washed three to four times before incubating in the binding buffer containing 1 μCi/ml of $^{45}Ca^{++}$ for 10 min. The membrane was rinsed with distilled water for 5 min, excess water was removed using Whatman No. 1 filter paper, and the membrane was dried overnight at room temperature. Autoradiographs of the $^{45}Ca^{++}$-labeled protein bands on the nitrocellulose membranes were obtained by exposure of the membranes to Kodak XAR-5 X-ray films for 12–20 h.

Dot-blot assay. To further delineate whether rhUG binds $^{45}Ca^{++}$ we used a dot-blot assay [45] using recombinant hUG with and without H-tag. Recombinant hUG with or without H-tag were dissolved in $NaHCO_3$ buffer (pH 7.4) and applied directly to Immobilon-P membrane using a dot-blot apparatus and air-dried. Membranes were soaked in a solution containing 60 mM KCl, 5 mM $MgCl_2$, and 10 mM imidazole–HCl (pH 6.8) and incubated in buffer containing $^{45}Ca^{++}$ (1 μCi/ ml) for 10 min, washed with imidazole buffer, dried, and autoradiographed as previously described [45].

Phospholipase A_2 assay. Phospholipase A_2 assays were performed according to the method described previously [30,31,33]. Briefly, the assay mixture contained 5 nM porcine pancreatic $sPLA_2$, 1 mM sodium deoxycholate, 10 μM [^{14}C]phosphatidylcholine, 100 mM Tris–HCl, pH 8.0, 100 mM NaCl, and 2 mM $CaCl_2$ in a total volume of 75 μl. [^{14}C]Phosphatidylcholine (500 pmol/reaction) was dried under N_2 for 45 min and then dissolved in freshly prepared 5 mM sodium deoxycholate (10 μl). The phosphatidylcholine–deoxycholate mixture was incubated at room temperature for 10 min and then mixed with two volumes of a mix solution containing 250 mM Tris–HCl, 250 mM NaCl, and 2.5 mM $CaCl_2$, pH 8. Forty microliters enzyme solution was mixed with an equal volume of inhibitor (i.e., rhUG) or H-tag hUG) or buffer and preincubated for 5 min at 37 °C. Reaction was started by the addition of 30 μl radioactive substrate to 20 μl reaction mixture. After 45 s at 37 °C, the reactions were stopped by addition of 50 μl of chloroform:methanol 2:1 (v/v), first followed by the addition of a mixture of 50 μl chloroform and 50 μl of 4 M KCl. After each of these three additions, the samples were mixed by vortexing for 30 s. Fifty microliters organic phase was spotted onto each lane of a prechanneled, preheated silica gel, and TLC plates were resolved in a chamber, saturated with petroleum ether:ethyl ether:glacial acetic acid 70:30:1 for 1 h. The plates were then developed in a chamber saturated with iodine vapor. TLC plates were analyzed using the Ambis Radioanalytical Imaging System Mark II (Ambis, CA) and the lipid bands from each reaction, co-migrating with arachidonic acid standard, were scraped and counted.

Results

Purification of wild-type and mutant rhUG with and without the H-tag

Both the wild-type and mutated rhUG were quantitatively recovered by using the Sephacryl S-200 superfine column, cation-exchange column, and Sephadex G-50 superfine column, respectively. As expected, the chromatographic behavior of these mutated proteins in the ionic exchange step was slightly different than that of wild-type rhUG (data not shown). Fractions containing the pure protein were pooled and lyophilized in small aliquots. We found that the fractions containing rhUG, eluted from Sephacryl S-200 column, must be extensively dialyzed against distilled water before the ion-exchange step is carried out to obtain reproducible binding of rhUG to the Econopak-S matrix. This step is required for both the wild-type as well as the mutated rhUG. In our hands, this simple purification scheme proved very reliable and reproducible, with yields of about 1.5 mg pure rhUG/g bacterial lysate (wet weight) for both the wild-type as well as the mutated rhUG. The homogeneity of the purified wild-type and mutated hUG was determined by silver staining of an overloaded gel, as shown in Figs. 1A and B. As expected, the use of β-mercaptoethanol disrupted the two disulfide bonds in the hUG dimer, as evidenced by the appearance of the monomeric hUG bands. Thus, the mutant rhUG is of the expected size and formed dimers similar to those of the wild-type hUG. The purified material is also immunoreactive to hUG antibody (Fig. 1C), as determined by Western blotting analysis.

Amplification of the rhUG coding sequence was performed by PCR using primers containing four histidine codons to introduce the H-tag, immediately preceeding the termination codon. The modified hUG cDNA-construct was electroporated into the *E. coli* strain BL21(DE 3) and the H-tag protein was purified to homogeneity by using Ni^{++} column chromatography (Amersham Pharmacia Biotech, Piscataway, NJ). Purified wild-type and mutant rhUG (MH_1, MH_2, and MH_3) did not contain reduced Cys residues, as determined by DTNB reaction (data not shown), and thus, are of dimeric conformation.

Phospholipase A_2 inhibitory properties of wild-type and mutated rhUG

To test the $sPLA_2$-inhibitory activity of the wild-type and the mutant rhUG, we used a previously established, phosphatidylcholine/deoxycholate mixed miceller assay [30,31,33]. It is clear that wild-type rhUG efficiently inhibits the $sPLA_2$ activity (Fig. 2). However, all three mutant proteins, MH_1, MH_2, and MH_3 failed to inhibit the $sPLA_2$ activity, as evidenced by the high level of

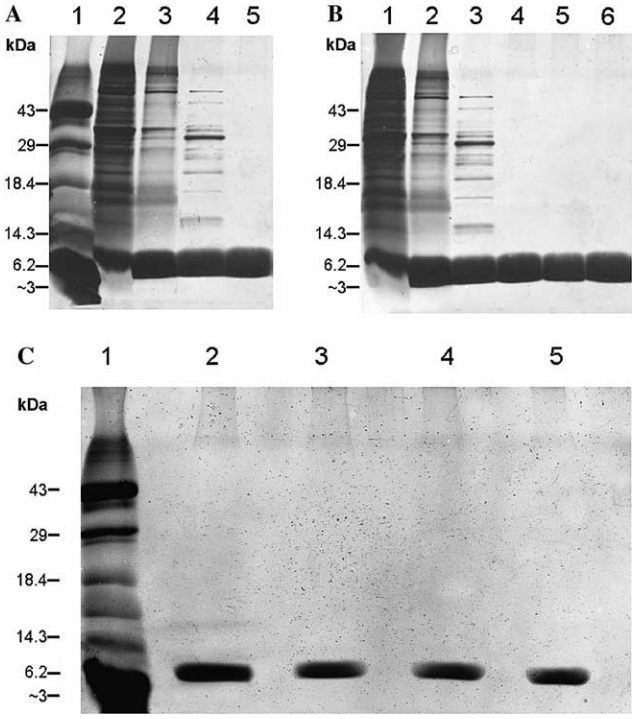

Fig. 1. Purification of human recombinant wild-type and mutant uteroglobin. (A) Wild-type protein: lane 1, molecular weight standards; lane 2, bacterial cell lysate; lane 3, proteins from Sephacryl S-200 column; lane 4, proteins from Econopak-S chromatography; lane 5, proteins from Sephadex G-50 chromatography. (B) Mutant proteins: lane 1, bacterial cell lysate; lane 2, proteins from Sephacryl S-200 column; lane 3, proteins from Econopak-S chromatography; lanes 4–6, proteins from Sephadex G-50 chromatography of MH_1, MH_2, and MH_3. (C) Immunoblot of purified proteins: lane 1, molecular weight standards; lane 2, rhUG; lane 3, MH_1; lane 4, MH_2; lane 5, MH_3.

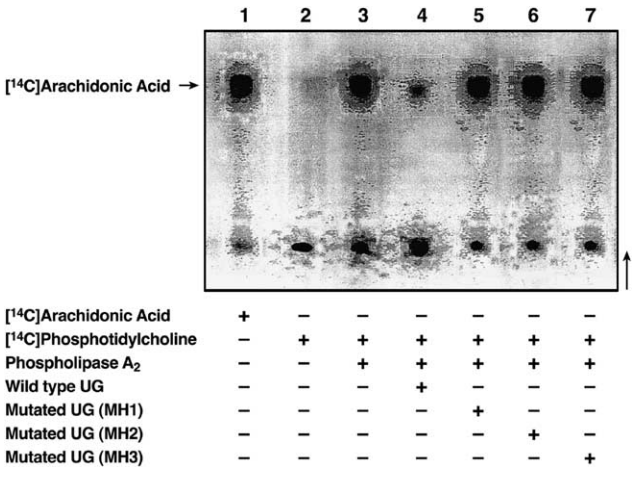

Fig. 2. Inhibition of phospholipase A_2 activity by wild-type and mutant uteroglobin. Lanes 1 and 2, [^{14}C]arachidonic acid and [^{14}C]phosphotidylcholine standards, respectively; lane 3, [^{14}C]phosphotidylcholine incubated with phospholipase A_2. Note the high level of arachidonic acid release; lanes 4–6, inhibition of phospholipase A_2 activity by wild-type and mutant rhUG.

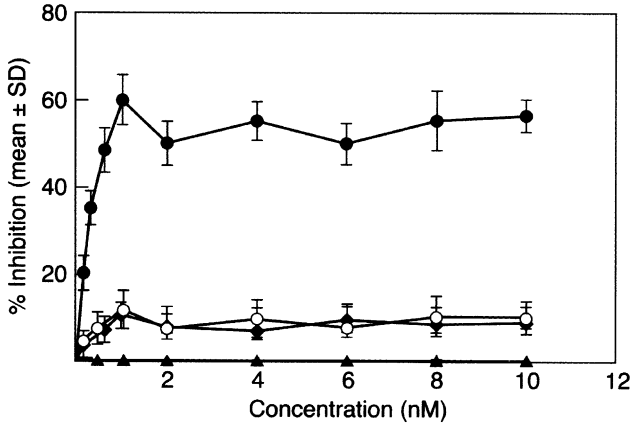

Fig. 3. Concentration-dependent inhibition of phospholipase A_2 by wild-type and mutant uteroglobin. Phospholipase A_2 inhibitory activities of wild-type rhUG, ●; phospholipase A_2 inhibitory activities of mutated uteroglobin (MH_1), ◆; phospholipase A_2 inhibitory activities of mutated uteroglobin (MH_2), ○; phospholipase A_2 inhibitory activities of lysozyme (control), ▲.

[^{14}C]arachidonic acid release from [^{14}C]phosphatidylcholine as substrate (Fig. 2). The lipid bands resolved by TLC were analyzed using an Ambis Radiological Imaging System, Mark II. We evaluated the concentration dependence and time course of $sPLA_2$ activity in addition to the effects of detergent concentrations. The inhibition of $sPLA_2$ activity by H-tag and non-H-tag-rhUG is approximately the same (data not shown).

In our assay conditions, we found that optimum deoxycholate concentration is 1.5 mM. As shown in Fig. 3, rhUG effectively inhibited the enzymatic activity of $sPLA_2$ in a dose-dependent manner. Under the conditions used, a maximum inhibition of about 62% of control was observed above 2 nM of wild type rhUG whereas the hUG mutants (i.e., Lys 43 to Glu, Asp 46 to Lys, and the double mutant) inhibited only 8–12% of control and non-specific proteins (lysozyme and BSA), used as negative controls, did not cause any detectable inhibition (Fig. 3).

Calcium-binding by H-tag rhUG

To determine whether $^{45}Ca^{++}$-binding is a property of rhUG or it is caused by the presence of H-tag, we tested purified rhUG with and without the H-tag by employing dot-blot and calcium overlay assays as previously described [42,45]. The results of the dot-blot and Ca^{++} overlay assays show that while H-tag rhUG avidly binds $^{45}Ca^{++}$ in a dose-dependent manner, rhUG without the H-tag failed to bind $^{45}Ca^{++}$ (Figs. 4A and B). Bovine calmodulin and chicken egg white lysozyme were used as positive and negative controls, respectively (Figs. 4A–C). As shown in Fig. 4D H-tag rhUG bound $^{45}Ca^{++}$ in a dose-dependent manner whereas even at the highest concentration of rhUG without the H-tag, $^{45}Ca^{++}$-binding was

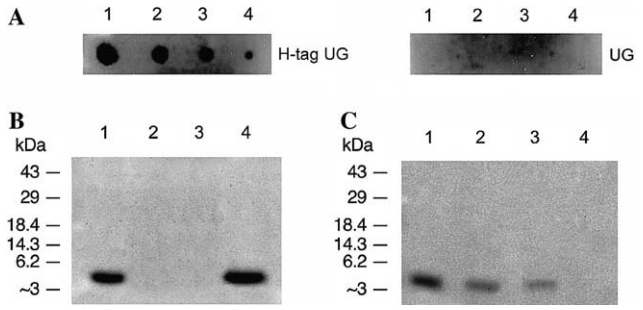

Fig. 4. Calcium-binding by recombinant human uteroglobin with and without the H-tag. (A) Dot-blot: left panel (H-tag rhUG), lane 1, calmodulin; lanes 2–4, 0.1 μg, 50 ng, and 25 ng H-tag rhUG, respectively. Right panel (rhUG without H-tag): lane 1, lysozyme; lanes 2–4, 0.1 μg, 50 ng, and 25 ng rhUG without H-tag, respectively. (B) Lane 1, calmodulin; lane 2, lysozyme; lane 3, uteroglobin without H-tag, and lane 4, uteroglobin with H-tag. (C) Concentration-dependent $^{45}Ca^{++}$-binding by recombinant H-tag-human uteroglobin. Lane 1, 0.1 μg H-tag rhUG; lane 2, 50 ng; lane 3, 25 ng H-tag rhUG; and lane 4, 0.1 μg rhUG without H-tag.

undetectable. These results suggest that the addition of H-tag to rhUG caused $^{45}Ca^{++}$-binding.

Discussion

In this study, we sought to establish: (a) whether specific amino acid residues in α-helix 3 of hUG are essential for the inhibition of $sPLA_2$activity and (b) whether rhUG has the ability to bind and presumably sequester Ca^{++}, required for $sPLA_2$ activation. The results of our experiments clearly demonstrate that Lys 43 and Asp 46 play crucial roles in the inhibition of $sPLA_2$ activity although the exact mechanism by which these amino acid residues confer $sPLA_2$-inhibitory activity to rhUG is not yet clear and deserves further investigation. Theoretically, it is possible that since these amino acid residues in α-helix 3 are exposed to the solvent, they are capable of participating in the binding of rhUG to mixed micellar substrates or in the formation of an rhUG/$sPLA_2$ complex that interferes with the interaction of $sPLA_2$ with the micellar substrate. The latter hypothesis is consistent with previous results obtained by chemical crosslinking and fluorescence spectroscopy of the only tyrosine residue in each of the UG monomers after interaction with $sPLA_2$ [33].

Recently, by using H-tag rhUG it has been reported [37] that the mechanism by which rhUG inhibits $sPLA_2$ activity is by binding and sequestering Ca^{++}, essential for the activation of $sPLA_2$. Several years ago, when we first demonstrated that natural UG, purified from the rabbit uterus, inhibits $sPLA_2$ activity [22], we entertained the possibility that this inhibition could be due to the sequestration of calcium by UG. However, upon repeated experimentation with purified UG we were unable to demonstrate any binding of $^{45}Ca^{++}$ by this protein. Since rabbit UG and hUG are virtually identical structurally, the results of Anderson et al. [37] may suggest that the binding of Ca^{++} observed in their experiments may be due to the presence of H-tag in the rhUG preparation. The results of our present investigation clearly demonstrate that Ca^{++}-binding is due to the presence of the H-tag in rhUG and that wild-type rhUG without H-tag does not bind Ca^{++}. Moreover, it is well known that $sPLA_2$ activation requires mM concentrations of Ca^{++}, whereas hUG inhibits $sPLA_2$ activity at nM concentrations. Thus, for any significant inhibition of $sPLA_2$ activity by hUG, it must bind Ca^{++} avidly and sequester it efficiently. For Ca^{++}-sequestration by hUG to be effective in inhibiting $sPLA_2$ activity, one has to assume that nM concentrations of rhUG bind mM amounts of Ca^{++}. However, the results of our binding assays fail to support this assumption in that only H-tag-rhUG but not rhUG without the H-tag can bind $^{45}Ca^{++}$. Furthermore, the results of structural studies with rabbit and human UGs did not reveal a Ca^{++}-binding motif in this protein [25–29]. Thus, the results of our studies demonstrating Lys 43 and Asp 46 in rhUG to be crucial for its $sPLA_2$-inhibitory activity and that rhUG without the H-tag fails to bind Ca^{++}, it is highly unlikely that sequestration of Ca^{++} by this protein is one of the mechanisms by which hUG inhibits $sPLA_2$ activity.

The possible physiological role of $sPLA_2$ inhibition by UG proteins in vivo remains to be clarified. It should be noted, however, that UG knockout mice have elevated plasma $sPLA_2$ activities [46]. The UG-deficient mice also manifest several phenotypes [46–49], suggesting a multifunctional nature of this protein. UG is present in alveolar and bronchial secretions and most likely protects the phospholipid component of lung surfactant by inhibiting the lung-specific $sPLA_2$ activity [50]. Phosphatidylcholine and phosphatidyl-ethanolamine are key components of the surfactant and we have recently reported that $sPLA_2$IA is expressed in the lung [50]. If activated, this enzyme is capable of degrading the phospholipids and facilitating the production of lipid mediators of inflammation. A significant population of premature human neonates with respiratory distress syndrome (RDS), a surfactant deficiency disease, go on to develop a chronic inflammatory lung disease known as bronchopulmonary dysplasia (BPD), after treatment with the surfactant. The fact that these neonates are not only surfactant-deficient, they also lack hUG, a potent inhibitor of $sPLA_2$ activity, suggests that activation of this enzyme in the absence of hUG may lead to the chronic inflammatory response. The presence of hUG in the secretions of a mature lung and its ability to protect surfactant phospholipids [51] strengthen the hypothesis that UG may play a protective role in the lung, in addition to maintaining an inflammation-free respiratory environment. Our results strongly suggest that amino acid residues, Lys 43 and Asp 46 in hUG α-helix 3, play crucial roles in this process.

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