Identification of the 32 kDa components of bovine lens EDTA-extractable protein as endonexins I and II

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EDTA-extractable protein (EEP) is a mixture of major lens membrane proteins with molecular masses ranging from 32 kDa to 40 kDa. These bind to the lens membrane in a Ca^{2+} -dependent manner. In the present study we have identified and purified two distinct 32 kDa components of EEP (designated as EEP 32-1 and EEP 32-2) from bovine lens that inhibit phospholipase A_2 activity. Both EEP 32-1 and EEP 32-2 bind to phospholipid-containing liposomes and actin filaments in a Ca^{2+} -dependent fashion. Immunochemical studies and two-dimensional electrophoreses demonstrate that the two proteins are distinct from one another. Both EEP 32-1 and EEP 32-2 are clearly different from calpactin (lipocortin) or its proteolytic fragments because they did not react with anti-[human placenta calpactin (lipocortin)] antibody. Our results also indicate that EEP 32-1 is very similar to endonexin I and that EEP 32-2 corresponds to endonexin II.

INTRODUCTION

The polypeptides of calf lens fibre membrane have been studied by various groups [1–8]. A group of proteins, the EDTA-extractable proteins (EEP), has been isolated on the basis of ability to bind to the lens fibre membranes in the presence of Ca^{2+} [1–8]. EEP represents a discrete group of different monomeric proteins with molecular masses of 32–33 kDa and 34–35 kDa. EEP appears to contain Ca^{2+} -binding sites with an apparent dissociation constant of $7.7 \mu\text{M}$ [9], and in the presence of Ca^{2+} EEP reassociates with EEP-depleted lens membrane and phospholipid-containing liposomes. Although the molecular properties of EEP have been investigated for several years, the physiological functions of the protein have not been defined.

A number of proteins have been purified on the basis of their properties of Ca^{2+} -dependent binding to cellular membranes or cytoskeletal fractions from various sources [10,11]. Two of these proteins, named calpactin I and calpactin II, are substrates for protein-tyrosine kinases of oncogene products [12–16] and growth-factor receptors [12–16]. The cDNAs coding calpactins I and II have been cloned and sequenced [17–19]. The calpactins were thought to be lipocortins, anti-inflammatory secreted proteins that inhibit phospholipase A_2 [15,17–19]. In addition, the calpactin/lipocortin family includes the following Ca^{2+} -binding proteins: pig intestine protein II [13,20], bovine liver endonexin I [21,22], p32.5 calelectrin from *Torpedo marmorata* [22], a 67 kDa protein from bovine aorta [23], human 67 kDa calelectrin [21,24], human placenta endonexin II [25] and human placenta anticoagulant protein [26,27].

Recent results on antibody cross-reactivity and functional properties have demonstrated that a 34–35 kDa

component of EEP (EEP 34) is identical with calpactin I (lipocortin II) [28]. It is also suggested that the 34 kDa protein might represent a biological activity in controlling the induction by fibroblast growth factor of growth and differentiation in the adult eye lens [29].

In the present paper we describe the purification of 32–33 kDa components of EEP (EEP 32) from bovine lens and compare their properties with those of the calpactin/lipocortin family proteins. The results indicate that the components of bovine lens EEP 32 closely resemble endonexins I and II in their biological activities, antibody cross-reactivity and physicochemical properties. The EEP 32 components are different from the 33 kDa core peptide of calpactins (lipocortins) in terms of antibody cross-reactivity.

MATERIALS AND METHODS

Materials

Q-Sepharose and S-Sepharose were products of Pharmacia Fine Chemicals. Hydroxyapatite was purchased from Bio-Rad Laboratories, and Ultrogel AcA-54 was purchased from LKB. Pig pancreatic phospholipase A_2 was a product of Boehringer, and [^3H]oleic acid was purchased from NEN. Horseradish-peroxidase-conjugated anti-(rabbit IgG) antibody was a product of Cappel, and nitrocellulose filter paper was purchased from Millipore. Acrylamide, bisacrylamide, SDS and Coomassie Brilliant Blue R-250 were purchased from Nakarai Chemicals. Phosphatidylserine, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol were purchased from Sigma Chemical Co. Ampholine was a product of LKB. Lysyl endopeptidase was purchased from Wako Pure Chemicals.

Abbreviation used: EEP, EDTA-extractable protein.

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Extraction of bovine lens EEP

Bovine lenses were obtained from a local slaughterhouse and frozen at -80°C until use. EEP was prepared from bovine lens by the procedure of Broekhuysse & Kuhlmann [3]. Briefly, decapsulated lenses were minced and homogenized in 50 mM-Tris/HCl buffer, pH 8.0, containing 1 mM- CaCl_2 with a Polytron homogenizer (setting 5) for 60 s. The Ca^{2+} -soluble fractions were separated from Ca^{2+} -insoluble material by centrifugation at 27000 *g* for 30 min. The insoluble fractions were washed in the same buffer and re-centrifuged three times. The fourth pellet was resuspended in the same buffer containing 7 M-urea and stirred for 20 min at 4°C . The solution was diluted with 3–7 vol. of the buffer and centrifuged at 27000 *g* for 30 min. The urea-treated particulate fraction was washed twice with the buffer. The final washed pellet was resuspended in 2 vol. of 50 mM-Tris/HCl buffer, pH 8.0, containing 5 mM-EGTA. The supernatant (EGTA extract) was obtained by centrifugation at 80000 *g* for 40 min.

Chromatographic purification

The EGTA extract was concentrated by a hollow-fibre concentrator (MN-3; Asahi-Kasei Co.) and dialysed against 10 mM-Tris/HCl buffer, pH 7.5. The dialysis residue was loaded on to a Q-Sepharose column (2.3 cm $\times$ 12 cm) pre-equilibrated with 10 mM-Tris/HCl buffer, pH 7.5. The column was washed with the same buffer until the eluent was protein-free and eluted with a linear NaCl gradient (0–0.75 M) in the same buffer. The 32 kDa protein fractions detected by SDS/PAGE were pooled and dialysed against 10 mM-potassium phosphate buffer, pH 6.8. The dialysis residue was applied to a hydroxyapatite column (1.5 cm $\times$ 10 cm). The column was washed with 150 ml of the buffer to elute unbound proteins. A 300 ml linear gradient of potassium phosphate buffer, pH 6.8 (10–300 mM), was employed to elute the 32 kDa protein. The 32 kDa protein fractions were pooled and further purified by gel filtration on an Ultrogel AcA-54 column (1.5 cm $\times$ 90 cm) pre-equilibrated and eluted with 10 mM-Tris/HCl buffer, pH 7.5, containing 0.1 M-NaCl. The elution volumes of all proteins were checked by SDS/PAGE, and the 32 kDa protein fractions were pooled. In some experiments gel-filtered 32 kDa protein was further fractionated by reverse-phase h.p.l.c. on Novapak C_{18} (Waters).

Protein purifications and production of antibodies

Calpactin I (lipocortin II), calpactin II (lipocortin I) and endonexin II (the anticoagulant protein) were purified from human placenta as described previously [13]. Briefly, the isolation scheme consisted of extraction of the Triton-insoluble fraction with EGTA-containing buffer followed by Q-Sepharose chromatography and S-Sepharose chromatography [30]. Rabbit skeletal-muscle actin was prepared by the method of Spudich & Watt [31] with further purification by gel filtration on Sephadex G-150 as previously described [32].

Antibodies to calpactin I (lipocortin II), calpactin II (lipocortin I), endonexin II and the 32 kDa component of EEP were produced by injecting 1 mg of each protein, emulsified in complete Freund's adjuvant, into rabbits intramuscularly. Booster shots were given three times at 2-week intervals with the antigens in the incomplete

adjuvant. The rabbits were bled 10 days after the last injections.

Analytical procedures

One-dimensional SDS/PAGE was performed as described by Laemmli [33] on 10% gels. Two-dimensional electrophoresis was performed by the method of O'Farrell [34] with 2% Ampholine (pH range 3–10) for the first dimension and a 10% polyacrylamide uniform gel for the second dimension. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 [35]. The following proteins were used as molecular-mass markers; phosphorylase *b* (94 kDa), BSA (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soya-bean trypsin inhibitor (20 kDa) and α -lactalbumin (14 kDa).

The electrophoretic transfer of proteins from SDS/PAGE gels or two-dimensional gels to nitrocellulose paper was performed for 2 h at 9 V/cm as described by Towbin *et al.* [36], with 40 mM-Tris/240 mM-glycine/5% (v/v) methanol, pH 8.3, as the transfer buffer. For immunodetection of the transferred proteins, the second antibody was linked to horseradish peroxidase. Antigen-antibody complexes were located by treating the bound peroxidase with diaminobenzidine and H_2O_2 [37].

Anti-(phospholipase A_2) activity was assayed as described by Rothhut *et al.* [38], with [^3H]oleic acid-labelled *Escherichia coli* membrane (3×10^5 d.p.m./nmol of phospholipid) as the substrate for pig pancreatic phospholipase A_2 .

F-actin-binding activity was measured as described previously [39]. The samples were mixed with rabbit skeletal-muscle F-actin (0.5 mg/ml) in 10 mM-Tris/HCl buffer, pH 8.0, containing 2 mM- MgCl_2 , 100 mM-KCl, 1 mM-ATP and 0.5 mM-dithiothreitol with 1 mM-EGTA or various concentrations of CaCl_2 . After incubation for 30 min at room temperature, F-actin and associated proteins were pelleted by centrifugation (100000 *g* for 20 min) in a Beckman Airfuge. Samples of the corresponding supernatants and pellets were analysed by SDS/PAGE. A phospholipid-containing-liposome-binding assay was carried out as described by Glenney *et al.* [16].

Bovine lens 32 kDa protein was digested with lysyl endopeptidase (enzyme/substrate ratio 1:400, by wt.) in 50 mM-ammonium bicarbonate buffer, pH 8.0, containing 0.01% SDS for 2 h at 37°C . The digest was acidified with formic acid to 20% (v/v). The resulting peptides were separated by reverse-phase h.p.l.c. on a Waters Novapak C_{18} column equilibrated with 0.1% (v/v) trifluoroacetic acid. Bound components were eluted with a 60 min gradient [0–90% (v/v) acetonitrile in 0.1% trifluoroacetic acid] at a flow rate of 1 ml/min. The fractions were freeze-dried and stored at -80°C for subsequent sequence analysis. Sequence analysis was performed on an Applied Biosystems 477A pulse-liquid sequencer in the presence of Polybrene. The amino acid phenylthiohydantoin derivatives were identified by an Applied Biosystems 120A analyser.

RESULTS

Purification

In agreement with earlier observations, we found that a discrete group of proteins can be extracted from bovine

Fig. 1. Purification of EEP 32-1 and EEP 32-2 from bovine lens

(a) SDS/PAGE analysis of EEP from bovine lens. Arrows indicate EEP components. Lane a, molecular-mass markers; lane b, crude EEP preparation. (b) SDS/PAGE analyses of selected fractions obtained during the purification. The arrow indicates EEP 32. Details of the conditions are described in the text. Lane a, EEP 32-1 (Q-Sepharose step); lane b, EEP 32-2 (Q-Sepharose step); lane c, EEP 32-1 (hydroxyapatite step); lane d, EEP 32-1 (Ultrogel AcA-54 step).

lens by using a urea/buffer washing procedure [3]. On 10% SDS/PAGE gels crude EEP consisted of 34 kDa, 33 kDa and 32 kDa polypeptides. Some slight contamination with crystallin could also be seen with this preparation (Fig. 1a, lane b). The crude EEP was subjected to Q-Sepharose chromatography. Several major protein peaks were detected. SDS/PAGE analyses indicated that the first peak, which was in the flow-through volume, contained a 34 kDa component of EEP as its major constituent. The second relatively broad peak, eluted at 0.2–0.3 M-NaCl, contained a 32 kDa protein as a major constituent (referred to as EEP 32-1; Fig. 1b, lane a). The third peak, eluted at 0.3–0.4 M-NaCl, contained a small amount of another 32 kDa protein (referred to as EEP 32-2; Fig. 1b, lane b) and contaminating crystallin. EEP 32-1 from the Q-Sepharose column was further purified by hydroxyapatite chromatography. EEP 32-1 was eluted as a single broad peak. The fractions with the highest purity, eluted at 170–210 mM-potassium phosphate, were pooled (Fig. 1b, lane c) and further purified by gel filtration on an Ultrogel AcA-54 column. EEP 32-1 was partially separated from contaminating 34 kDa protein, and the fractions with the highest purity were pooled (Fig. 1b, lane d). Approx. 5 mg of the purified protein was obtained from 500 g of bovine lens by this procedure. At this stage EEP 32-1 was over 90% pure.

Gel filtration

Analytical gel filtration of purified EEP 32-1 on a TSK G3000SW h.p.l.c. column revealed a Stokes radius of 2.15 ± 0.1 nm, indicative of a molecular mass of about 30 kDa for a globular protein (results not shown).

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis of purified EEP 32-1 revealed that the protein consists of several isoelectric variants present in unequal amounts (Fig. 2).

Fig. 2. Two-dimensional gel electrophoresis of purified EEP 32-1 (a) and partially purified EEP 32-2 (b)

The approximate isoelectric points of major spots are pI 5.9, pI 5.7 and pI 5.4. EEP 32-2, which was partially purified by Q-Sepharose chromatography, was also analysed by this technique. The preparation consists of two major isoelectric variants (pI 5.2 and pI 5.0) with some contamination with EEP 32-1.

Phospholipid-binding properties of EEP 32-1 and EEP 32-2

The Ca^{2+} -dependent binding of EEP 32-1 and EEP 32-2 to phospholipids was investigated by measuring the ability of the proteins to co-sediment with phospholipid vesicles when subjected to high-speed centrifugation. In the absence of Ca^{2+} , neither EEP 32-1 nor EEP 32-2 co-pelleted with phospholipid-containing liposomes [liposomes containing cholesterol (0.5 mg/ml), phosphatidylserine (0.25 mg/ml) and phosphatidylcholine (0.25 mg/ml)]. At 20 μM -, 40 μM -, 60 μM -, 80 μM - and 100 μM - Ca^{2+} , 11%, 48%, 79%, 95% and 97% respectively of the added proteins were in the pellet (results not shown). These experiments showed that binding of EEP 32-1 and EEP 32-2 to phospholipids is specific and requires the presence of Ca^{2+} . We examined the phospholipid-specificity of EEP 32-1 and EEP 32-2 binding to liposomes (Fig. 3). Only a small fraction of EEP 32-1 and EEP 32-2 was found in the pellet in the presence of cholesterol or phosphatidylcholine. In contrast, the proteins co-pelleted with phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine. These results indicated that EEP 32-1 and EEP 32-2 are very similar in their phospholipid-specificity.

Fig. 3. Phospholipid-specificity of EEP 32-1 and EEP 32-2 binding to liposomes

Details of the conditions are described in the text. Incubations were done in the presence of 0.1 mM- CaCl_2 . After high-speed centrifugation, samples of the corresponding supernatants (S) and pellets (P) were analysed on 10% SDS/PAGE gels. Lane 1, cholesterol; lane 2, phosphatidylserine; lane 3, phosphatidylinositol; lane 4, phosphatidylcholine; lane 5, phosphatidylethanolamine.

F-actin binding

Since calpactin I (lipocortin II) and calpactin II (lipocortin I) have been shown to bind to actin filaments at high Ca^{2+} concentration, we examined whether EEP 32-1 and EEP 32-2 have the same F-actin-binding activity *in vitro*. Neither EEP 32-1 nor EEP 32-2 bound to actin filaments, as judged by a co-sedimentation assay in the presence and in the absence of Ca^{2+} (Fig. 4). Neither protein co-sedimented with F-actin in the absence of Ca^{2+} , but increasing amounts of the proteins were detected in the pellet with increasing Ca^{2+} concentrations. At 0.10 mM-, 0.25 mM-, 0.50 mM-, 1.0 mM- and 2.0 mM- Ca^{2+} , 10%, 15%, 31%, 39% and 41% respectively of the added proteins were in the pellet (results not shown).

Inhibitory activity towards phospholipase A_2

As shown in Fig. 5, increasing amounts of EEP 32-1 and EEP 32-2 lead to a progressive decrease in phospholipase A_2 activity. Both EEP 32-1 and EEP 32-2 and calpactin I (lipocortin II) displayed a similar inhibitory curve over a broad concentration range.

Immunological cross-reactivity

The functional and chemical similarities of EEP 32-1, EEP 32-2, endonexin I, endonexin II and calpactins (lipocortins) suggest a close relationship between these

Fig. 4. F-actin-binding properties of EEP 32-1 (a) and EEP 32-2 (b)

Details of the conditions are described in the text. Incubations were done in the presence of 0.1 mM- CaCl_2 (C) or 1 mM-EGTA (E). After high-speed centrifugation, samples of the corresponding supernatants (S) and pellets (P) were analysed on 10% SDS/PAGE gels. Arrows indicate EEP 32-1 or EEP 32-2. The arrowhead indicates actin.

proteins. We raised antisera in rabbit to EEP 32-1 after final purification by SDS/PAGE. In addition, we used antibodies against calpactin I (lipocortin II), calpactin II (lipocortin I) and endonexin II (the anticoagulant protein) to test for the immunological cross-reactivity (Fig. 6). The anti-(EEP 32-1) antibody was specific for this protein, showing no detectable cross-reaction in Western blots with EEP 32-2 and human placenta endonexin II. In contrast with the results, antibody to endonexin II recognized EEP 32-2 as well as human placenta

Fig. 5. Inhibition of phospholipase A_2 activity by EEP 32-1, EEP 32-2 and calpactin I

Phospholipase A_2 activity was measured in the presence of inhibitor proteins as described in the Materials and methods section. Enzyme activities are expressed as percentages of the value obtained in the absence of inhibitor proteins. Symbols: $\circ$, EEP 32-1; $\blacktriangle$, EEP 32-2; $\bullet$, calpactin I.

Fig. 6. Immunological comparisons of EEP 32-1 and EEP 32-2

(a) Lane 1, Coomassie Blue-stained gel of a mixture of human placenta calpactins I and II; lane 2, immune-gel replicate with anti-(calpactin I) antibody; lane 3, immune-gel replicate with anti-(calpactin II) antibody. (b) Lanes 1, EEP 32-1; lanes 2, EEP 32-2. Panel a shows Coomassie Blue-stained proteins. Panels b–e show immune-gel replicates of the proteins with anti-(EEP 32-1) antibody (panel b), anti-(endonexin II) antibody (panel c), anti-(calpactin I) antibody (panel d) and anti-(calpactin II) antibody (panel e).

endonexin II itself. Antibodies to human placenta calpactins I and II were specific for the proteins, showing no detectable cross-reaction in the blots with EEP 32-1 and EEP 32-2. In a Western-blotting experiment with a crude EEP preparation separated on an SDS/PAGE gel, antiserum to EEP 32-1 reacted only with the 32 kDa band, whereas antiserum to calpactin I reacted with both the 34 kDa band and the 33 kDa band (results not shown). Since calpactin I preparations from other sources routinely contain 33 kDa proteolytic fragments [16,40], we infer that the 33 kDa band is generated by proteolysis during the purification procedure.

Partial sequence analysis of EEP 32-1

Preliminary attempts to sequence intact EEP 32-1

Fig. 7. Partial amino acid sequence of EEP 32-1

(a) Lysyl endopeptidase-cleaved peptide map of EEP 32-1. Details of the conditions are described in the text. (b) Comparison of amino acid sequences of EEP 32-1, bovine endonexin I [41], pig protein II [20] and human endonexin II [27,42].

were unsuccessful, indicating that the *N*-terminal end of the protein may be blocked. The protein was digested with lysyl endopeptidase, and cleavage products were separated by reverse-phase h.p.l.c. on a C_{18} column. Fig. 7 shows a peptide map generated for the protein. Cleavage products from EEP 32-1 were further analysed by protein sequence analysis. For the cleavage peptides, we have identified the corresponding region within endonexins I and II, on the basis of the high degree of sequence homology between these proteins [20,27,41,42]. EEP 32-1 gave a sequence that was a 25/25 match with the sequence of pig protein II or bovine endonexin I [20,41].

DISCUSSION

Intracellular Ca^{2+} has been shown to play a major role in the regulation of diverse events such as secretion, membrane transport, contraction and cell division [43]. Recently, members of a group of Ca^{2+} -binding proteins have been purified on the basis of their property of Ca^{2+} -dependent reversible binding to cellular membranes, phospholipid-containing liposomes or cytoskeletal fractions from various sources (for reviews see refs. [10] and [11]). The molecular masses of most of these Ca^{2+} - and phospholipid-binding proteins fall within two groups, one between 67 and 73 kDa and the other between 32 and 40 kDa. The latter group contains two related proteins, calpactin I (lipocortin II) and calpactin II (lipocortin I), which have been extensively studied [10–19]. In this family, besides calpactins or lipocortins, several related proteins have been purified from different sources and reported as 35 kDa calcimedin [31,44], p32.5 calelectrin [22], protein II [13,20], endonexin I [21,22], endonexin II [25] and the anticoagulant protein [26,27]. On the basis of their published characteristics, these proteins are generally very similar to each other. However, on the basis of their behaviour during ion-exchange chromatography and their isoelectric points, these proteins appeared to be divided into at least two different groups. One group contains protein II, p32.5 calelectrin and endonexin I, whereas the other group contains

endonexin II and the anticoagulant protein. Amino acid sequence data also suggested that endonexin I (protein II) and endonexin II (the anticoagulant protein) are distinct [20,25,27,41,42].

The EEP is a major extrinsic protein component of lens membrane. EEP is composed of two major polypeptides with apparent molecular masses of 32–33 kDa and 34–35 kDa. EEP is more prevalent in the cortical areas of the lens and was found to decrease with aging [45]. Recently Russell *et al.* [28] reported that the 34–35 kDa component of EEP is identical with calpactin I (lipocortin II) and contains phosphotyrosine residues. They also showed that the 32–33 kDa component of EEP may represent the proteolytic fragment of calpactin I.

In the present study we have identified and purified two distinct 32 kDa components of EEP from bovine lens that inhibit phospholipase A_2 activity. These proteins bind to membrane and other particulate fractions in the presence of Ca^{2+} . The extractability is very similar to those of 34 kDa EEP and other calpactin/lipocortin-related proteins from various tissues [1–9]. Present studies also showed that the 32 kDa EEP is separated into two different peaks during ion-exchange chromatography. A major portion of the 32 kDa EEP (i.e. EEP 32-1), which was eluted in the earlier fractions, was further purified by hydroxyapatite chromatography and gel filtration. A minor portion of the 32 kDa EEP (i.e. EEP 32-2), which corresponded to 20–30% of the major peak, was eluted in the later fractions on the ion-exchange column. Our immunochemical studies and two-dimensional electrophoreses demonstrated that the two protein peaks (EEP 32-1 and EEP 32-2) are distinct from each other. These results strongly support the hypothesis that the 32 kDa EEP is a mixture of proteins rather than a single protein. Both EEP 32-1 and EEP 32-2 are clearly different proteins from calpactin I (lipocortin II) and calpactin II (lipocortin I) or their proteolytic fragments because they did not react with antiserum against human placenta calpactin I or calpactin II; on the other hand lens 34 kDa component of EEP reacted with the antibody. Our results also indicate that EEP 32-2 corresponds to endonexin II (the anticoagulant protein) by immunochemical criteria. Behaviour during ion-exchange chromatography and partial amino acid sequence data indicated that EEP 32-1 is identical with endonexin I or protein II [13,20,25,27,41,42].

Our data present clear evidence that EEP 32-1 and EEP 32-2 display phospholipase A_2 -inhibitory activity, F-actin binding and liposome binding similar to those of lipocortins. Although it has not directly been reported, the phospholipase A_2 -inhibitory activity of the 34 kDa EEP can be expected. Because EEP binds both to lens fibre membrane and to purified cytoskeletal proteins in the presence of Ca^{2+} , they may provide links in membrane-cytoskeletal interactions. Furthermore, EEP may provide a link between the steps in the cell division and differentiation processes. Blanquet *et al.* [29] proposed that the possible regulation of phospholipase A_2 activity due to fibroblast-growth-factor-stimulatable dephosphorylation of 34 kDa EEP may be involved in signal transduction of fibroblast growth factor in the adult eye lens. Whatever the molecular mechanism of the phospholipase A_2 -inhibitory action and F-actin-binding activity of EEP 32, our observation raises some intriguing questions concerning the biological significance of EEP.

Further investigation should allow us elucidate the true activities of EEP in the lens.

REFERENCES

1. Broekhuysse, R. M. & Kuhlmann, E. D. (1974) *Exp. Eye Res.* **19**, 297–302
2. Bloemendal, H., Vermorken, A. J. M., Kibbelaar, M., Bunia, I. & Beneditti, E. L. (1977) *Exp. Eye Res.* **24**, 413–415
3. Broekhuysse, R. M. & Kuhlmann, E. D. (1978) *Exp. Eye Res.* **26**, 305–320
4. Bouman, A. A., de Leeuw, A. L. M. & Broekhuysse, R. M. (1981) *Exp. Eye Res.* **32**, 491–500
5. Bouman, A. A., de Leeuw, A. L. M. & Broekhuysse, R. M. (1981) *Exp. Eye Res.* **32**, 299–308
6. Bouman, A. A., de Leeuw, A. L. M. & Broekhuysse, R. M. (1981) *Exp. Eye Res.* **33**, 309–314
7. Raaij, A. J. M., de Leeuw, A. L. M. & Broekhuysse, R. M. (1985) *Exp. Eye Res.* **40**, 477–487
8. Raaij, A. J. M., Feijen, A. & Snoek, G. M. (1987) *Exp. Eye Res.* **45**, 215–225
9. van den Eijnden-van Raaij, A. J. M., de Leeuw, A. L. M. & Broekhuysse, R. M. (1985) *Curr. Eye Res.* **4**, 789–792
10. Klee, C. B. (1988) *Biochemistry* **27**, 6645–6653
11. Crompton, M. R., Moss, S. E. & Crompton, M. J. (1988) *Cell* **55**, 1–3
12. Fava, R. A. & Cohen, S. (1984) *J. Biol. Chem.* **259**, 2636–2645
13. Gerke, V. & Weber, K. (1984) *EMBO J.* **3**, 227–233
14. Glenney, J. R., Jr. (1985) *Lett.* **192**, 79–82
15. De, B. K., Misono, K. S., Lukas, T. J., Moczowski, B. & Cohen, S. (1986) *J. Biol. Chem.* **261**, 13784–13792
16. Glenney, J. R., Jr., Tack, B. & Powell, M. A. (1987) *J. Cell Biol.* **104**, 503–511
17. Huang, K.-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., Burne, C., Frey, A., Hession, C., McGray, P., Sinclair, L. K., Chow, E. P., Browning, J. L., Ramachandran, K. L., Tang, J., Smart, J. E. & Pepinsky, R. B. (1987) *J. Biol. Chem.* **262**, 7639–7645
18. Saris, C. J. M., Kristensen, T., Tack, B. F., Glenney, J. R., Jr. & Hunter, T. (1986) *Cell* **46**, 201–212
19. Wallner, B. P., Mattaliano, R. J., Hession, C., Cate, R. L., Tizard, R., Sinclair, L. K., Foeller, C., Chow, E. P., Browning, J. L., Ramachandran, K. L. & Pepinsky, R. B. (1986) *Nature (London)* **320**, 77–80
20. Weber, K., Johnson, N., Plessman, U., Van, P. N., Söling, H.-O., Ampe, C. & Vandeckerckove, J. (1987) *EMBO J.* **6**, 1599–1604
21. Geisow, M. J. & Walker, J. H. (1986) *Trends, Biochem. Sci.* **11**, 420–423
22. Geisow, M. J., Fritsche, U., Hexam, J. M. & Johnson, T. (1986) *Nature (London)* **320**, 636–638
23. Martin, F., Derancourt, J., Capony, J.-P., Colote, S. & Cavadore, J.-C. (1987) *Biochem. Biophys. Res. Commun.* **145**, 961–968
24. Sudhof, T. C., Slaughter, C. A., Leznich, I., Barjon, P. & Reynolds, G. A. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 664–668
25. Schlaepfer, D. D., Mehlman, T., Burgess, W. H. & Haigler, H. T. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6078–6082
26. Funakoshi, T., Heimark, P. L., Hendrickson, L. E., McMullen, B. A. & Fujikawa, K. (1987) *Biochemistry* **26**, 5572–5578
27. Iwasaki, A., Suda, M., Nakao, H., Nagoya, T., Saino, Y., Arai, K., Mizoguchi, T., Sato, F., Yoshizaki, H., Hirata, M., Miyata, T., Shidara, Y., Murata, M. & Maki, M. (1987) *J. Biochem. (Tokyo)* **102**, 1261–1273

28. Russell, P., Zelenka, P., Martensen, J. & Reid, T. W. (1987) *Curr. Eye Res.* **6**, 533–538
29. Blanquet, P. R., Paillard, S. & Courtois, Y. (1988) *FEBS Lett.* **229**, 183–187
30. Pepinsky, R. B., Tizard, R., Mattaliano, R. J., Sinclair, L. K., Miller, G. T., Browning, J. L., Chow, E. P., Burne, C., Huang, K.-S., Pratt, D., Wachter, L., Hession, C., Frey, A. Z. & Wallner, B. P. (1988) *J. Biol. Chem.* **263**, 10799–10811
31. Spudich, J. A. & Watt, S. (1971) *J. Biol. Chem.* **256**, 4866–4871
32. MacLean-Fletcher, S. & Pollard, T. D. (1980) *Biochem. Biophys. Res. Commun.* **96**, 18–27
33. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
34. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
35. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2613
36. Towbin, H., Staehelin, T. H. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
37. Graham, R. C. & Karnovsky, M. J. (1966) *J. Histochem. Cytochem.* **14**, 291
38. Rothhut, B., Russo-Marie, F., Wood, J., DiRosa, M. & Flower, R. J. (1983) *Biochem. Biophys. Res. Commun.* **117**, 878–884
39. Kobayashi, R., Bradley, W. A., Bryan, J. & Field, J. B. (1983) *Biochemistry* **22**, 2463–2469
40. Huang, K.-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., Burne, C., Frey, A., Hession, C., McGray, P., Sinclair, L. K., Chow, E. P., Browning, J. L., Ramachandran, K. L., Tang, J., Smart, J. E. & Pepinsky, R. B. (1986) *Cell* **46**, 191–199
41. Hamman, H. C., Gaffey, L. C., Lynch, K. R. & Creutz, C. E. (1988) *Biochem. Biophys. Res. Commun.* **156**, 660–667
42. Kaplan, R., Jaye, M., Burgess, W. H., Schlaepfer, D. D. & Haigler, H. T. (1988) *J. Biol. Chem.* **263**, 8037–8047
43. Kretsinger, R. H. (1980) *CRC Crit. Rev. Biochem.* **8**, 119–174
44. Moore, P. B. & Dedman, J. R. (1982) *J. Biol. Chem.* **257**, 9663–9667
45. Bouman, A. A., de Leeuw, A. L. M. & Broekhuysen, R. M. (1981) *Exp. Eye Res.* **31**, 495–504

Received 16 June 1989/22 September 1989; accepted 1 November 1989