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Further Characterization of Four Lipocortins From Human Peripheral Blood Mononuclear Cells

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Four calcium and phospholipid binding proteins purified from mononuclear cells were characterized for PKC and EGF phosphorylation, actin binding capacity, and partial tissue distribution. Those named 35K, 32K, and 73K are equivalent, respectively, to lipocortin III, endonexin II and the 67 kDa calelectrin; 36K is a fragment of 73K. After purification, 35K and 73K were phosphorylated by protein kinase C in vitro but 36K nor 32K were not. None were phosphorylated by the epidermal growth factor receptor kinase in vitro; 73K bound F-actin in a calcium-dependent manner, whereas 35K, 36K, and 32K did not. Using Western blotting analysis, 32K and 73K were detected in high amounts in human lymphocytes, monocytes, liver, and placenta and in rat adrenal medulla; but 32K was not detected in polymorphonuclear cells, and 36K and 35K were detected in high amounts only, respectively, in human blood lymphocytes and polymorphonuclear cells. Thus, 32K and 73K appear to have a wide tissue distribution, whereas 35K has a much more restricted distribution.

Key words: phospholipase A_2, lipocortins, phosphorylations, actin-binding

Purification and cloning of two phospholipase A_2 (PLA_2) inhibitory proteins [1,2] have shown that they belong to a new family of calcium-and phospholipid-binding proteins (CPBP) named *annexins* or *lipocortins* [3,4,5]. Calelectrins [6], calpactins [reviewed in 7], calcimedins [8], some chromobindins [9], lipocortins I and II, proteins I, II [10], endonexin II [11], a human lymphocyte p68 [12], a placental anticoagulant protein (PAP) [13], an inhibitor of blood coagulation (IBC) [14], and some PLA_2

Abbreviations used: ATP, adenosine triphosphate; DTT, dithiothreitol; CPBP, calcium and phospholipid binding protein; EDTA, ethylene diamine tetraacetic acid; EGF, epidermal growth factor; EGTA, ethyleneglycol-bis-(β-amino-ethyl ether) N,N,N′,N′ tetraacetic acid; IBC, inhibitor of blood coagulation; kDa, kilodalton; Mr, molecular weight; PAP, placental anticoagulant protein; PBS, phosphate-buffered saline; PKC, protein kinase C; PLA_2, phospholipase A_2; PMSF, phenylmethyl-sulfonylfluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA, trichloro acetic acid.

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inhibitory proteins [15] are all members of this family. Identification of CPBPs has arisen from various fields of investigations, such as studies on intracellular calcium regulation of cytoskeletal functions, intracellular substrates for the tyrosine kinase activity of growth factor receptors and oncogen products, membrane movements such as exocytosis, and cellular control of inflammatory response by corticosteroids. CPBP properties, when analyzed, are similar: they bind calcium and acidic phospholipids in a calcium-dependent manner [reviewed in 16]; their amino acid sequences are constituted of the repeat of an homologous sequence of about 70 amino acids containing a highly conserved "consensus sequence" [17]; they inhibit PLA_2 in vitro probably indirectly by sequestering the acidic phospholipidic substrate [15,18,19]. Some have been shown to bind, in a calcium-dependent manner, actin and spectrin [7,10,11,20,21,22,23], and some appear to be substrates for tyrosine kinases [7] and/or protein kinase C [see 7,11,13,24–29].

Thus, CPBPs are suggested to act as links between membranes and the submembranous cytoskeleton, and these properties could be regulated by intracellular calcium and phosphorylation processes. Nevertheless, their physiological role and the significance of their diversity are still unknown. They are derived from a common ancestral gene that evolved through a series of gene duplication events. CPBPs include proteins of an apparent Mr of about 70 kDa that are probably identical and of several proteins in the 30–40 kDa range that are not yet fully distinguishable. This group of 30–40 kDa proteins share common properties and can be differentiated from one another essentially by their amino acid sequence [1,2,11–13,24,30] or by their characteristic migration on two-dimensional electrophoresis [9]. Four have been clearly identified: lipocortin I (identical to calpactin II), lipocortin II (identical to calpactin I heavy chain), protein II (similar to endonexin and chromobindin 4), and endonexin II, which is identical to the coagulation inhibitor (PAP or IBC) and to lipocortin V [30]. Western blot analysis or two-dimensional electrophoresis suggest that CPBPs have differential cell and tissue repartition [1,16,30,31].

The authors recently characterized, in blood human mononuclear cells, six calcium and phospholipid binding proteins that all inhibit PLA_2 in vitro: one has an apparent Mr of 73 kDa and five have apparent Mr's between 30 and 40 kDa. Two of them are lipocortin I and II. The four others were purified according to Rothhut et al. [15] characterized and named 73K, 32K, 35K, and 36K. These differ from endonexin and lipocortin I and II. Partial amino acid sequencing has shown that 32K and 35K are identical to endonexin II (IBC, PAP, and lipocortin V) (B. Rothhut, personal communication) and lipocortin III, respectively. 73K and 36K recognized equally antibodies directed against either 36K, the 67 kDa calelectrin [6], the bovine aorta 67 kDa actin-binding protein [20]; 36K appeared to be the N-terminal part of the 73K.

We report here a further characterization of these four proteins: two of them (35K and 73K) were substrates for PKC in vitro. None was substrate for epidermal growth factor (EGF) receptor tyrosine kinase, and only 73K bound F-actin in a calcium-dependent manner. Their partial tissue and cell localization showed that 32K and 73K were widely distributed and always detected in high amounts except in polymorphonuclear cells, where 32K is absent. Proteins 35K and 36K were found only as important components of, respectively, polymorphonuclear cells and lymphocytes from human blood.

MATERIALS AND METHODS

Cells, Tissues, and Materials

Human blood mononuclear cells and polymorphonuclear cells were obtained from the Blood Transfusion Center (Hôtel-Dieu, Paris, France). Human liver was a gift from Dr. Jeanne Etiemble and Dr. M.A. Buendia at Pasteur Institute and rat adrenal medullae from Dr. A. Parini (INSERM U7, Paris). Bovine aortic actin purified according to Cavadore et al. [32] and F-actin glutaraldehyde stabilized and linked on sepharose-4B was a gift from Dr. J.C. Cavadore (Montpellier, France). [γ-^{32} P]ATP (20 mCi/ml) were from Amersham. Recombinant lipocortin I was a gift from J.L. Browning (Biogen Research Corporation).

Purification of 35K, 36K, 32K, and 73K Lipocortins

Mononuclear cells were washed in phosphate-buffered saline (PBS), resuspended in 10 mM HEPES, 5 mM EDTA, 100 mM NaCl, 200 μM PMSF, 1,000 P.I.U./ml aprotinin (one P.I.U., peptidase inhibitory unit inhibits the activity of a Schwert and Takenaka unit of trypsin), pH 7.6 and frozen at −80°C until used. Lipocortins were isolated by calcium precipitation and EGTA solubilization and the 35K, 36K, 32K, and 73K were purified by one-step chromatography and analyzed in SDS-PAGE and isoelectric focusing as previously described [15]. Bovine lung 35K and 73K have also been purified using the same method.

Western Blotting Analysis

Monocytes and lymphocytes were separated from human blood mononuclear cells using Sepracell-MN (Sepratech corporation, Oklahoma City, OK) as described by the manufacturer. Washed cells or jagged frozen tissues were lysed in RIPA buffer containing 140 mM NaCl, 10 mM Tris-HCl, pH 7.6, 10 mM EDTA, 1% Nonidet P-40, 0.1% SDS, 1 mM PMSF, 1000 *PIU/ml* aprotinin, sonicated five times 10 s and mixed with SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Equal amounts of proteins (100 or 200 μg) from different extracts or 0.5 or 1 μg of the purified proteins were subjected to SDS-PAGE, electrotransferred onto nitrocellulose sheets, and submitted to Western blotting as previously described [15] using polyclonal antibodies raised against 35K, 36K, or 32K according to [33].

In Vitro Phosphorylation Assay by Protein Kinase C and EGF-Receptor Kinase

In vitro phosphorylation was performed using protein kinase C purified by J.M. Pelosin from bovine or rat brain as described by Pelosin [34]. Briefly, each purified protein (1.8 μg/assay) was incubated for 10 min with 2.5 IU of bovine brain PKC in the presence of 5×10^{-5} M adenosine triphosphate (ATP), 1.8×10^6cpm of [γ-^{32}P]-ATP (4 nmole), 750 μM $CaCl_2$, 20 μg/ml phosphatidylserine, and 0.8 μg/ml dioleyl glycerol. In vitro EGF receptor phosphorylation was done using both A-431 membranes and EGF according to Cochet et al. [35]. Proteins were separated by SDS-PAGE containing 10% polyacrylamide, and [γ-^{32}P] incorporation was revealed by a 16 h autoradiography with Kodak X-OMat films. The time course of PKC phosphorylation experiments were performed using 1 μg of either bovine lung 35K, 73K from human mononuclear cells, or human recombinant lipocortin I, 4 IU of rat brain PKC, 5×10^{-5} M ATP, 1.8×10^6 cpm of [γ-^{32}P]-ATP, 10 μM $MgCl_2$, 750 μM$CaCl_2$, 150 μg/ml phosphatidylserine, and

5 μg/ml dioleyl glycerol. Phosphorylated proteins were resolved in SDS-PAGE, and the Coomassie-blue stained phosphorylated proteic bands were cut out from the gel and subjected to liquid scintillation spectrophotometry. Using the second PKC phosphorylation conditions, the three proteins were subjected to 15 min phosphorylation in both the presence and absence of phospholipids.

F-Actin Binding Assay

F-actin binding assays were performed using two different techniques: 1) By chromatography using F-actin glutaraldehyde stabilized and linked to sepharose-4B according to Martin et al. [36]; briefly, protein samples (10 μg/assay) equilibrated in buffer A (25 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM dithiothreitol (DTT) and 1 mM $CaCl_2$) were allowed to stay in contact with Immobilized F-actin for 16 hours at 4°C, and then unbound material was washed out by six column volumes of buffer A at a flow rate of 2ml/h, and bound proteins were eluted with buffer B (25 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM DTT, and 2 mM EGTA); 2) F-aortic actin mixed with pure protein in actin polymerizing buffer (10 mM imidazole/HCl, pH 7.5, 1 mM NaCl, 1 mM DTT, 0.3 mM ATP, 100 mM KCl, and 2 mM $MgCl_2$, with either 2 mM $CaCl_2$ or 2 mM EGTA) [20]. In these experiments, the molar ratio of actin and protein was 5:1. Some additional experiments have been performed without KCl, because some CPBPs have been reported to bind F-actin only without KCl in the sample buffer [21]. After a 1 h incubation with gentle agitation at room temperature, the samples were centrifuged at 100,000 xg for 30 min in a Beckman airfuge. The supernatant and pellet were separated. Proteins of supernatants and chromatographic samples were concentrated by precipitation with 10% (W/V) TCA, 5 min in ice, centrifuged 5 min at 10,000 xg, and washed twice for 5 min with cold acetone. All samples then were submitted to SDS-PAGE and Coomassie-blue staining as described in [15]. F-actin binding of bovine lung 73K as a function of Ca^{2+} concentration was performed with EGTA/$CaCl_2$ buffers, using a molar ratio of F-actin and protein of 10:1 and with 2 h incubation times.

RESULTS

Proteins 35K, 36K, 32K, and 73K were purified from mononuclear cells according to Rothhut et al. [15]. They were eluted from MonoQ column at 0.13M, 0.18M, 0.20M, and 0.27M of NaCl, respectively. They all were homogeneous as judged by SDS-PAGE and isoelectric focusing. Polyclonal antibodies directed against 35K, 32K, and 36K were raised and appeared to be very specific when tested by Western blot experiments with pure 35k, 32K, 36K, 73K, and lipocortins I and II. After a long film exposure, only anti-35K antibodies showed very weak cross-reaction with the five other lipocortins (not shown).

Phosphorylation Experiments

As shown in Figure 1, PKC phosphorylated in vitro both 35K and 73K but neither 36K nor 32K. In Western blot experiments, 1 μg of pure 35K did not react with antibodies raised against other CPBPs that are PKC substrates—calpactin I, calpactin II, or endonexin—whereas 1 μg of control immunoreactive proteins showed a strong recognition. A time course PKC phosphorylation of 35K, 73K, and the control recombinant lipocortin I have been performed to determine the extent of phosphorylation. As

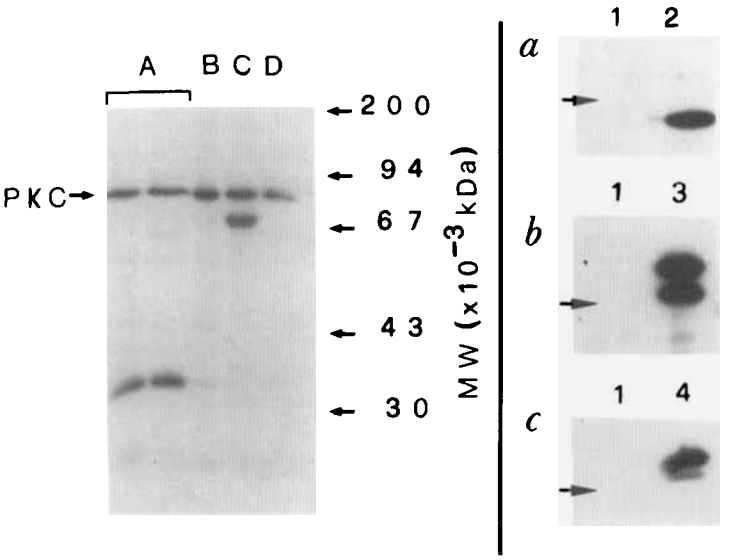

Fig. 1. In vitro phosphorylation of 35K (lanes A), 36K (lane D), 32K (lane B), and 73K (lane C) (1.8 μg of each protein/assay) with 2.5 IU of PKC. Incorporation of [γ-^{32}P] was visualized after a 10% polyacrylamide SDS-PAGE and autoradiography for 16 h. Arrows on the left show the autophosphorylated PKC position and on the right the migration position of molecular weight standards.

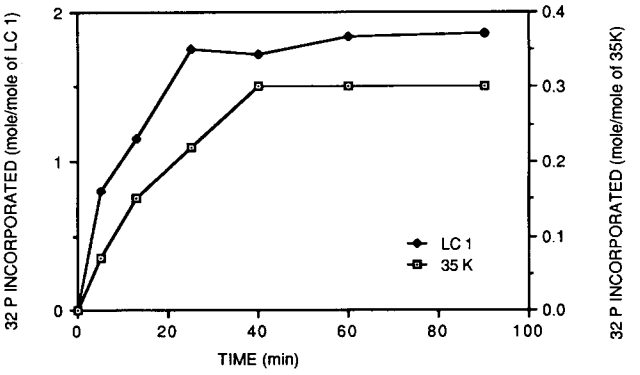

Fig. 2. Time course of in vitro PKC phosphorylation of lipocortin I and 35K; 1 μg of each protein are subjected to PKC phosphorylation for 5, 13, 25, 40, and 90 min. Phosphorylation extent is expressed in moles of [γ-^{32}P] incorporated per protein mole.

shown in Figure 2, PKC phosphorylation of recombinant lipocortin I and 35K plateaued at 30 and 40 min, respectively, with a stoichiometry of 1.8 and 0.3 mole of incorporated [γ-^{32}P] per mole of protein. The extent of lipocortin I PKC phosphorylation is in accordance with the previously described [γ-^{32}P] incorporation, which was of two moles per mole of protein [37,38]. In contrast, 73K incorporation was not more than 0.05 [γ-^{32}P] mole per mole of protein, suggesting that it is a poor substrate for this enzyme. The presence of phospholipids increased the extent of PKC phosphorylation for the three proteins (Table I), but lipocortin I phosphorylation appeared to be much more phospho-

TABLE I. Stoichiometry of [γ^{32}P] Incorporation During PKG Phosphorylation of Lipocortin I, 35K, and 73K in the Presence or Absence of Phospholipids*

Protein	[γ-^{32}P] incorporated (mole/mole of protein)		Factor of multiplication of [γ-^{32}P] incorporated in the presence of phospholipids
	Without phospholipids	With phospholipids	
LCI	0.033	1.170	35.4
35K	0.017	0.100	5.9
73K	0.026	0.056	2.1

*Phosphorylation time was 15 min.

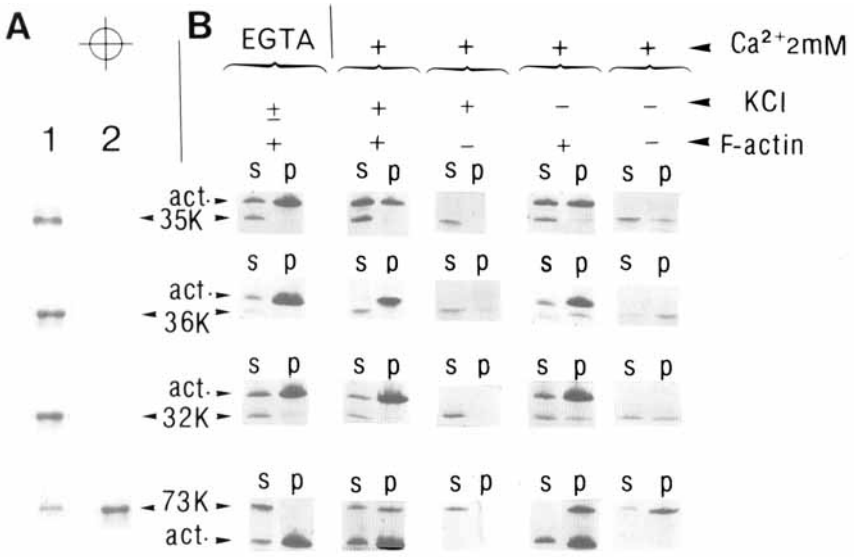

Fig. 3. F-actin-binding assays analysed by SDS-PAGE and staining with Coomassie blue. **A:** Affinity chromatography. Column 1 is the wash in buffer A (1 mM $CaCl_2$) and column 2, elution with buffer B (2 mM EGTA). **B:** Experiments with F-actin and centrifugation as described in Materials and Methods. s, supernatant; p, pellet. The presence of F-actin, KCl, $CaCl_2$, or EGTA are mentioned on the top. Bands corresponding to actin (act.) and each protein are indicated by arrows.

lipid-dependent than the two others. The same four proteins were submitted to in vitro phosphorylation by EGF receptor kinase: none incorporated [γ-^{32}P] when the kinase was activated by EGF addition, even in the presence of 1 mM $CaCl_2$ (data not shown), whereas EGF receptor autophosphorylation was detected.

F-Actin Binding Experiments

The four purified proteins were submitted to F-actin binding assay using an affinity chromatography on F-actin linked sepharose 4B column (Fig. 3A); 35K, 36K, and 32K were washed out from the column with buffer A, and no bound proteins were eluted with buffer B (with 2 mM EGTA). Only the 73K showed a Ca^{2+}-dependent binding on immobilized actin and was eluted from the column with buffer B.

In experiments using bovine F-actin and centrifugation in airfuge at 100,000 × g for 30 min (Fig. 3B), no protein cosedimented with actin in the presence of EGTA with

or without KCl. In the presence of 2 mM $CaCl_2$ and 100 mM KCl, only 73K cosedimented with F-actin and was not found in the pellet in absence of actin. In presence of 2 mM $CaCl_2$ and no KCl, the four proteins cosedimented with F-actin, but they also self-sedimented in the presence of 2 mM $CaCl_2$ and in the absence of F-actin and KCl. Thus, they were precipitable in the presence of Ca^{2+} and low ionic strength. In these low ionic strength conditions, in order to dissociate nonspecific sedimentation from specific F-actin binding, the same experiments were performed using twice as much actin (molar ratio of actin and protein of 10:1); but even in these experimental conditions, 35K, 36K, and 32K were not pelleted in greater amount with F-actin (data not shown). The same experiments performed with 1 mM $CaCl_2$ and no KCl showed a decrease of the self-sedimentation of 35K, 36K, and 32K; however, no specific actin binding was ever observed (not shown). The amount of 73K sedimented in the F-actin pellet increased as a function of calcium concentration. No significant F-actin binding was observed at 0.1 mM Ca^{2+}. At 0.5, 1, 2, and 5 mM Ca^{2+} and in the presence of 100 mM KCl, 15%, 20%, 40% and 60% of 73K were in the pellet as mesured by densitometry after SDS-PAGE and Coomassie blue staining. In the same conditions, 73K self-sedimentation is constant at about 10–15%.

Tissue and Cell Distribution of the 35K, 36K, 32K, and 73K Lipocortins

The presence of 35K, 36K, 32K, and 73K in rat adrenal medulla and human liver, placenta, monocytes, and lymphocytes was examined by Western blotting experiments using specific polyclonal antibodies (see Fig. 4). In the liver and placenta, 32K was detected in high amounts as a single reacting band comigrating with pure 32K and in a similar amount in both lymphocytes and monocytes (not shown). It was detected in a lesser amount in adrenal medulla and was not found in polymorphonuclear cells. To detect the presence of 73K and 36K in the five homogenates tested, the serum directed

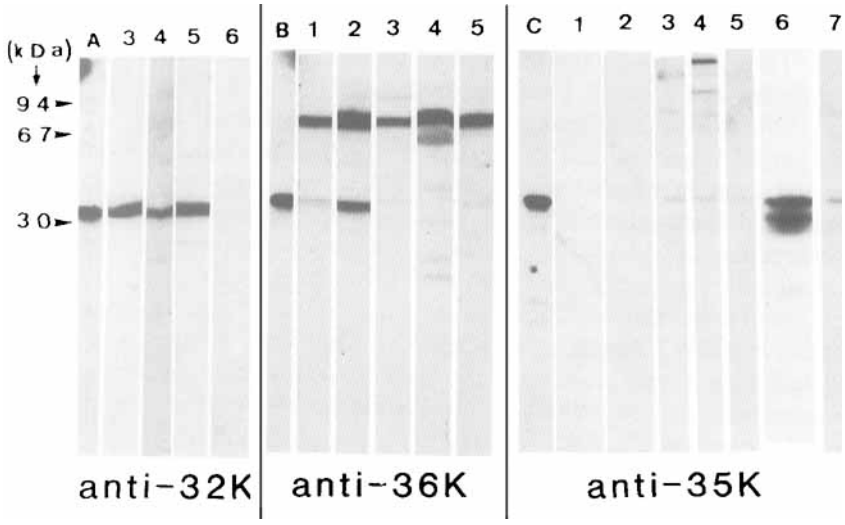

Fig. 4. Western blot detections of 32K, 35K, 36K, and 73K in monocytes (lane 1) lymphocytes (lane 2), placenta (lane 3), adrenal medulla (lane 4), liver (lane 5), blood polymorphonuclear cells (lane 6), and bovine lung (lane 7). **A, B, C** show the control immunoreactivity of, respectively, 1 μg of 32K, 36K, and 0.5 μg of 35K. Except for the 35K distribution experiment were 100 μg of homogenate proteins were used.

against 36K, which reacts equally with these two proteins, was used; 73K was found in high amounts in all lysates but mainly in lymphocytes and adrenal medulla. Only lymphocytes had an abundant immunoreactive 36K. Anti-35K antibodies recognized a weak band, comigrating with standard pure 35K, in adrenal medulla, liver, placenta, and lung but not in lymphocytes and monocytes. Other larger proteins (75 to 100 kDa) cross-reacted with this serum, but their relationship with the 35K is unknown. Interestingly, two immunoreactive proteins of 32 and 35 kDa were detected in very high amounts in polymorphonuclear cells; 32 kDa might be a proteolytic fragment of 35K.

DISCUSSION

Previous work in our laboratory has shown that human peripheral blood mononuclear cells contain six different calcium and phospholipid-binding proteins that all inhibit PLA_2 in vitro. Two of these are lipocortin I and II (or calpactin II and I), whose actin-binding properties, phosphorylation capacities, and tissue distribution have been well characterized. We have attempted to analyse similar properties for the other four proteins.

Other CPBPs have been reported to bind F-actin only in the absence of KCl [21–23] or in a Ca^{2+} concentration-dependent manner [11]. Nevertheless, no data were given concerning the behaviour of these proteins in the absence of F-actin. Using either an affinity chromatography assay or sedimentation experiments in presence of 100 mM KCl, 73K bound F-actin in a Ca^{2+}-dependent manner, whereas the other three proteins were unable to bind F-actin in these experimental conditions. Although all four proteins cosedimented with F-actin in low ionic strength conditions (no KCl), the cosedimentation of 35K, 36K, and 32K could not be increased with twice more F-actin. When 1 mM $CaCl_2$ was used, the self-sedimentation of these three proteins was decreased, but no specific F-actin binding was ever detected. The amount of phospholipids in the samples of the four purified proteins was determined with phosphate titration [39,40]. The estimated content of phospholipids during F-actin experiments was less than 0.02 nmole/ml. Thus, no phospholipid-dependent precipitation that could impair protein-actin association would occur. These results suggest that 35K, 36K, and 32K did not specifically bind to F-actin, at least in the in vitro used conditions.

For partial tissue and cell localization of the four proteins, we selected adrenal medulla and liver, in which the different CPBPs have been well characterized by two-dimensional electrophoresis [9,30], and placenta, which has been often used to isolate these proteins [2,13,14]. In addition, we decided to determine from which type of mononuclear cells (lymphocytes or monocytes) the proteins originated. Both 32K and 73K were found to be relatively abundant in all homogenates tested except polymorphonuclear cells, in which 32K was not detected. By comparing the intensity of the reaction obtained with 1 μg of pure protein and the band recognized in the whole extract, it was possible to evaluate the amount of both proteins to about 0.5% of total cellular proteins, as previously described [2]. Their presence in all tissues tested is consistent with a wide distribution of these two proteins. The lower amount of 32K in adrenal medulla could not be attributed to species differences as rat and human lipocortins V (identical to 32K) share 90% amino acid sequence homology [30]. This partial tissue repartition analysis of 32K is in accordance with a similar study showing that lipocortin V is absent from neutrophils. In contrast, this group did not detect lipocortin V in both thymus and spleen, whereas it was found in peripheral blood monocytes and lymphocytes, but they detected

lipocortin V mRNA in rat peritoneal macrophages. When purified from mononuclear cells, 50 to 100 μg of 32K is always obtained from 10^{10} cells; 36K was detected in high amount only in lymphocyte homogenates, which suggests that 36K might be present in physiological condition in intact lymphocytes. Nevertheless, we cannot exclude the possibility that it is derived from 73K by proteolytic cleavage during cellular lysis.

The protein 35K, which is identical to lipocortin III, was absent from lymphocytes and monocytes and present in very low amount in liver, adrenal medulla, and placenta, but it is an abundant component of polymorphonuclear cells (neutrophils), representing about 0.5% of the total proteins. In this experiment, no cross-reaction was detectable with 0.5 μg control of the other CBPBs, suggesting that the tissue reactivities are specific for 35K and not due to nonspecific cross-reactivities. A CPBP, named PAP III [41], whose partial amino acid sequence establishes identity with lipocortin III and 35K, has been purified from human placenta. This agrees with 35K detection in this tissue. Recently, Sato et al. [42] studied a tissue distribution of a 33 kDa guinea pig neutrophils CPBP. They did not detect it in lymphocytes, macrophages, liver, heart, brain, and kidney but found it abundant in neutrophils. They showed, using immunohistochemical fluorescence, that the 33 kDa protein detected in lung and spleen is localized only in the neutrophils of these tissues. The low amount of 35K in lung, placenta, and liver may also correspond to residual neutrophils in these tissues. However, 35K is weakly detected in adrenal medulla and also in isolated bovine adrenal cortex (A. Alfsen, personal communication). Thus, if 35K distribution agrees with an identity with the 33 kDa guinea pig neutrophil protein, it seems localized not only in neutrophils. It should be noted that the partial amino acid sequence of the 33 kDa guinea pig neutrophils protein is different from lipocortin III and almost identical to rat lipocortin I [30]. These results suggest that the purified 35K that obtained does not originate from mononuclear cells, but it certainly does originate from residual neutrophils. This may explain the observed variations in the amount of pure 35K that we obtained.

The number of CPBPs is growing, but their physiological action in cells is not yet understood. Among them, calpactin I heavy chain seems to be the best substrate for PKC in vivo and in vitro [25,28,29,31], but calpactin II and protein II (probably identical to endonexin and chromobindin 4) and a 67 kDa CPBP are also substrates in vitro for this kinase [24,28,43]. We report here that 35K and 73K are also phosphorylated by PKC in vitro, but neither 32K nor 36K *are*. Thus, the ability of these proteins to be phosphorylated by serine-threonine kinases seems to be a widespread property. Tyrosine phosphorylation has been described only for calpactin I (substrate for the oncogen product of $pp60^{src}$) and for calpactin II (substrate of the EGF-receptor kinase). None of our four proteins were substrates for the EGF-receptor kinase. The role of phosphorylations either by PKC or by tyrosine kinase on the properties of the proteins remains controversial. Nevertheless, it is suggested that the physiological action of CPBPs could be regulated by phosphorylation/dephosphorylation. In such cases, tissue repartition of CPBPs could be the consequence of their kinase(s) specificity.

In conclusion, the reported 73K properties (F-actin binding and phosphorylation by PKC) are in accordance with other results reported for CPBPs of about 70 kDa [20,43]. These results, together with immunoreactivities and amino acid sequences [12,20,44], suggest that they might be the same unique mammalian protein. We also report that a new lipocortin (35K) is a substrate for PKC in vitro. Finally, F-actin binding, at least in vitro, does not seem a general property for CPBPs.

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