A 32 kDa lipocortin from human mononuclear cells appears to be identical with the placental inhibitor of blood coagulation

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A 32 kDa protein isolated from human mononuclear cells is a member of the lipocortin family, a new group of Ca^{2+} -dependent lipid-binding proteins thought to be involved in the regulation of phospholipase A_2 , in exocytosis and in membrane–cytoskeleton interactions. Purification of this protein was based on its ability to associate with membrane phospholipids in a Ca^{2+} -dependent manner and its capacity to inhibit purified phospholipase A_2 from pig pancreas. Using immunological detection, we show that it is present in various cells involved in the inflammatory and coagulation processes. We present extensive amino acid data that strongly suggest that this protein is identical with a recently described inhibitor of blood coagulation, with endonexin II and with lipocortin V. Sequence alignment with other known proteins show a significant degree of homology with lipocortins I and II, the substrates of the epidermal-growth-factor receptor tyrosine kinase and the oncogene pp60^{src} tyrosine kinase respectively, and with protein II. The possible physiological role of this 32 kDa lipocortin is discussed.

INTRODUCTION

Lipocortins were first described as glucocorticoid-induced secreted proteins present in many cell types and tissues, including rabbit neutrophils (Hirata *et al.*, 1980), rat macrophages (Blackwell *et al.*, 1980), rat renal-medullary interstitial cells (Cloix *et al.*, 1983), mouse and bovine thymus (Gupta *et al.*, 1984), human fibroblasts (Errasfa *et al.*, 1985) and even in rabbit platelets (Touqui *et al.*, 1986). The predominant form identified was a 40 kDa protein whose major activity was to inhibit purified soluble phospholipases A_2 from pig pancreas and snake venom. Other species, of 70 kDa, 30 kDa and 15 kDa, were identified and shown to be immunologically related to the 40 kDa protein (Hirata *et al.*, 1982; Rothhut *et al.*, 1983), and therefore the proposed name of lipocortin was adopted (Di Rosa *et al.*, 1984). The anti-inflammatory effect of glucocorticoids was in part attributed to the synthesis and/or release of these proteins [for reviews see Flower *et al.* (1984) and Hirata (1984)]. Moreover, a regulatory pathway controlling phospholipase A_2 activity, involving protein tyrosine kinase (Hirata *et al.*, 1984) and protein kinase C (Touqui *et al.*, 1986), has been proposed. Evidence for the presence of lipocortins as extracellular proteins came from their first purification to homogeneity from the peritoneal fluid of dexamethasone-treated rats (Pepinsky *et al.*, 1986). The same group purified and cloned two human phospholipase A_2 inhibitors, named lipocortins I and II and exhibiting 50% homology (Wallner *et al.*, 1986; Huang *et al.*, 1986). The compared immunological properties and sequences also showed a complete identity with another family of related Ca^{2+} -dependent phospholipid and actin-binding proteins: calpactin II or p35 and the

heavy chain of calpactin I or p36 (Glenney, 1986; Gerke & Weber, 1984). These intracellular proteins are known as the major targets of tyrosine kinases: the epidermal-growth-factor receptor for calpactin II (Fava & Cohen, 1984) and the retrovirally coded tyrosine kinase pp60^{src} for p36 (Radke & Martin, 1979; Erikson & Erikson, 1980; Cooper & Hunter, 1983). In addition, protein kinase C phosphorylates both proteins *in vitro* (Isacke *et al.*, 1986; Khanna *et al.*, 1986), and p36 *in vivo* (Gould *et al.*, 1986).

These proteins belong to a new class of Ca^{2+} - and phospholipid-binding proteins found in the literature under the following names: calelectrin (Walker, 1982), endonexin (Südhof *et al.*, 1984), p68/p70 (Owens *et al.*, 1984), proteins I and II (Gerke & Weber, 1984), synexin (Creutz *et al.*, 1978), chromobindin (Creutz, 1981), calcimedin (Moore & Dedman, 1982) and synhibin (Pollard & Scott, 1982). All these proteins share a characteristic structure consisting of four, or eight (p68), segments of 70–80 amino acid residues showing a significant degree of homology, containing a highly conserved consensus sequence of 17 amino acid residues (Saris *et al.*, 1986; Kristensen *et al.*, 1986; Brugge, 1986; Weber & Johnsson, 1986; Geisow *et al.*, 1986; Weber *et al.*, 1987; Crompton *et al.*, 1988). It has been proposed to give to this new class of Ca^{2+} - and phospholipid-binding proteins the name 'annexins' (Geisow, 1986; Geisow & Walker, 1986).

In recent work (Rothhut *et al.*, 1987) we have shown the existence of members of this family in cells involved in the inflammatory process: human peripheral-blood mononuclear cells. The predominant member was a 32 kDa protein, purified and identified as a lipocortin. It was different from lipocortins I and II because antibodies

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against these proteins did not cross-react with the 32 kDa protein (C. Coméra, B. Rothhut & F. Russo-Marie, unpublished work). Nevertheless results showing a cross-reactivity with a monoclonal antibody (BF26) raised against a rat lipocortin (Rothhut *et al.*, 1984) provided evidence for this protein to be a true member of the lipocortin family. The pure protein tested on soluble phospholipases A₂ (from pig pancreas and *Naja naja* venom) showed a dose-dependent inhibitory effect restricted to negatively charged phospholipids, but only at low substrate concentrations. These data suggested that inhibition of the enzyme by lipocortin involved its Ca²⁺-dependent binding to negatively charged phospholipids (Davidson *et al.*, 1987; Haigler *et al.*, 1987; Aarsman *et al.*, 1987). An alternative, in order to explain the results at high substrate concentration, was the possible formation of an inactive enzyme-inhibitor complex, dissociated by an excess of phospholipids (Aarsman *et al.*, 1987). The protein was also able to inhibit purified cellular phospholipases A₂ from rat platelets and rat liver mitochondria as well as endogenous phospholipase A₂ in an assay using thymocytes (Rothhut *et al.*, 1987) or monocytes (C. Coméra, B. Rothhut & F. Russo-Marie, unpublished work).

In the present paper we study the cellular distribution of this new 32 kDa lipocortin in different cells involved in the inflammatory process. It is a Ca²⁺-dependent phospholipid-binding protein. We report extensive amino acid sequence data of this 32 kDa protein. It appears that it is completely identical with a recently described inhibitor of blood coagulation (Funakoshi *et al.*, 1987a, b; Iwasaki *et al.*, 1987), with another protein from human placenta, namely endonexin II (Schlaepfer *et al.*, 1987; Haigler *et al.*, 1989), and with lipocortin V (Pepinsky *et al.*, 1988). We also provide evidence that this protein is an inhibitor of blood coagulation and propose a mechanism involving a Ca²⁺-dependent interaction with phospholipids and biological membranes.

METHODS

Purification of the 32 kDa lipocortin

The 32 kDa protein was purified as described previously (Rothhut *et al.*, 1987).

Anti-(32 kDa lipocortin) serum

An antiserum against purified 32 kDa lipocortin was prepared in rabbits by using the lymph-node immunization procedure (Sigel *et al.*, 1983).

Western-blot analysis

Washed cells were lysed in RIPA buffer (10 mM-Tris/HCl buffer, pH 7.6, containing 140 mM-NaCl, 10 mM-EDTA, 1% Nonidet P40, 0.1% SDS, 1 mM-phenylmethanesulphonyl fluoride and 200 i.u. of aprotinin/ml), and mixed with SDS sample buffer [80 mM-Tris/HCl buffer, pH 8.0, containing 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 3% (w/v) SDS and 0.1% Bromophenol Blue]. Equal amounts of protein (100 µg) from the different extracts were subjected to SDS/polyacrylamide-gel electrophoresis (12.5% gel), electrotransferred on to nitrocellulose sheets and subjected to Western-blot analysis as described by Towbin *et al.* (1979) except that ¹²⁵I-Protein A (100 000 c.p.m./ml) was used for immunological detection.

Phospholipid-binding assays

(a) **Binding on *Escherichia coli* membranes.** The binding of lipocortin was assessed by using the same conditions as used to test phospholipase A₂-inhibitory activity (Rothhut *et al.*, 1983), except in one assay where the inhibitor was preincubated with the substrate and the reaction was initiated by the addition of the enzyme (1 h). After the reaction was stopped, the samples were centrifuged (13 000 *g* for 5 min), and the radioactivity of a portion of each of the supernatants containing hydrolysed [³H]oleic acid was counted by liquid-scintillation spectrometry. The percentage inhibition was calculated by comparison with the control of basal release of [³H]oleic acid without phospholipase A₂ as the blank and the maximal enzyme activity in absence of inhibitory protein as the 100% value. The pellets were washed once with incubation buffer and analysed by SDS/polyacrylamide-gel electrophoresis and Coomassie Blue staining. Controls included a reaction in which substrate was omitted (a) and an assay in which EGTA was substituted for Ca²⁺ in the incubation buffer (b).

(b) **Binding on immobilized phospholipids.** Phosphatidylserine and phosphatidylinositol immobilized on polyacrylamide (Uchida & Filburn, 1984) were equilibrated in 10 mM-Hepes/NaOH buffer, pH 7.4, containing 100 mM-KCl, 0.5 mM-dithiothreitol and either 1 mM-CaCl₂ or 2 mM-EGTA. Portions (1 µg) of the pure protein were incubated with the bound ligands (50 µl) for 2 h at 20 °C and then centrifuged (5000 *g* for 5 min), and the supernatants and pellets were each mixed with an equal volume of electrophoresis SDS sample buffer. A sample (one-fiftieth) of each fraction was separated by SDS/polyacrylamide-gel electrophoresis (10–15% gradient gels) and stained with the silver reagent by using the Phast System from Pharmacia.

Coagulation assays

Activated partial thromboplastin time, expressed in seconds, was determined according to the procedure given by the manufacturer (Organon) with or without the 32 kDa lipocortin at the final concentrations indicated. Citrated normal plasma was prepared from six healthy subjects: blood (9 vol.) was drawn on trisodium citrate at 3.8% (1 vol.) and centrifuged at 2000 *g* for 30 min.

Specific coagulation studies of intrinsic coagulation Factors VIII, IX, XI and XII were done by a conventional method (Caen *et al.*, 1975): diluted normal plasma (1/10, 1/20 and 1/40), containing 100 µg of lipocortin/ml (final concentration) or not, was added to commercially available plasma depleted in coagulation Factors VIII, IX, XI or XII. Activated partial thromboplastin time was determined for all dilutions as indicated above.

Amino acid sequence analysis

The purified protein (1.4 nmol) from human mononuclear cells was carboxymethylated with 0.32 mg of iodo[1-¹⁴C]acetic acid in 100 µl of 0.5 M-Tris/HCl buffer, pH 8.5, containing 6 M-guanidinium chloride under Ar, after incubation for 2.5 h in the presence of 100 µg of dithiothreitol. After 40 min reaction, the mixture was acidified with 100 µl of acetic acid, diluted with 1.8 ml of 0.1% (v/v) trifluoroacetic acid and filtered on a Waters Associates Sep-Pak C₁₈ cartridge. The latter

was washed with 20 ml of 0.1 % trifluoroacetic acid and the protein was eluted with 3 ml of 60 % (v/v) acetonitrile in 0.1 % trifluoroacetic acid. The yield, as indicated by amino acid analysis, was 50 %. For trypsin digestion, the blocked protein was dissolved into 90 μ l of 0.1 M- NH_4HCO_3 /0.11 mM- CaCl_2 , pH 7.75. After 4 h at 37 °C, the sample was acidified with 20 μ l of formic acid and dried. Peptide separation was achieved on a Spheri 5 RP-8 column (100 mm $\times$ 2.1 mm) from Brownlee Laboratories, eluted at 0.2 ml/min with a gradient from 0 to 100 % between 5 % acetonitrile in 0.05 % trifluoroacetic acid (solvent A) and 90 % acetonitrile in 0.05 % trifluoroacetic acid (solvent B).

All the peptides were sequenced with an Applied Biosystems 470A gas-phase sequencer with on-line identification on an Applied Biosystems 120A h.p.l.c. system. The amounts undergoing degradation varied from 5 to 320 pmol.

RESULTS

We have first described this protein in peripheral-blood mononuclear cells. In order to evaluate its expression in different types of cells, we have screened by Western blotting diverse cells involved in the inflammatory and coagulation processes, as well as a histiocytic lymphoma cell line U937.

Fig. 1 shows the Western-blot analyses of lysates from different cell types with a rabbit polyclonal antibody raised against the 32 kDa protein. Under these experimental conditions it was undetectable in human erythrocytes (lane 9); most of the other cells tested [human platelets (lane 2), mouse megakaryocytes (lane 3), endothelial cells (lane 4), U937 cells (lane 5),

Fig. 1. Western-blot analysis of extracts from various cells with a rabbit polyclonal antibody raised against the 32 kDa protein from peripheral-blood mononuclear cells

The results show the relative distribution of the protein within the different cell types and the comparison can only be qualitative. The different cell extracts are as follows: human platelets (lane 2), mouse megakaryocytes (lane 3), human umbilical endothelial cells (lane 4), U937 cells (lane 5), human lymphocytes (lane 6), human monocytes (lane 7) and human erythrocytes (lane 9). Lane 1 represents the pure reduced 32 kDa protein, and lane 8 shows the same protein under non-reducing conditions. The arrows indicate the molecular-mass standards and are, from the top, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soya-bean trypsin inhibitor and α -lactalbumin.

lymphocytes (lane 6), monocytes (lane 7)] gave a single band migrating at the same position as the pure protein (lane 1). The same band was also detected in HL-60 cells, a human promyelocytic-leukaemia cell line, but was undetectable in human neutrophils (results not shown). Under non-reducing conditions (lane 8), another band at 68 kDa was detected; it could originate from the dimerization of the molecule as reported by Funakoshi *et al.* (1987a). In control experiments the pre-immune rabbit serum was tested on the same cellular extracts and no cross-reacting bands were ever detected (results not shown).

To compare this protein with the other members of the Ca^{2+} /lipid-binding protein family, we investigated the ability of the purified 32 kDa protein to bind to phospholipids.

In a first set of experiments we analysed the capacity of the purified protein to bind to *E. coli* membranes by using the phospholipase A_2 assay described in the Methods section. Results in Fig. 2 show that the protein binds to the phospholipidic substrate. This binding is Ca^{2+} - and dose-dependent and is well correlated with the phospholipase A_2 inhibition determined in the same assays. By using this procedure we could also analyse the effects of preincubation conditions. The phospholipase A_2 inhibition (half-maximal) obtained when 1 μ g of inhibitor was preincubated with the enzyme increased to 75 % after preincubation of 1 μ g of lipocortin with the substrate (Fig. 2, compare lanes g and h). This increased inhibition is also correlated with an increased protein binding to the lipid membranes. The relative amounts of the Coomassie Blue-stained 32 kDa bands were determined by densitometric scanning (results not shown). This method confirmed the increase in the amount of protein bound to the *E. coli* membranes.

In a second set of experiments, we investigated the

Fig. 2. Binding of the 32 kDa lipocortin to *E. coli* membranes

Increasing concentrations of the protein were tested for phospholipase A_2 -inhibitory activities and binding to the oleic acid-labelled substrate as described in the Methods section. The amounts of 32 kDa protein were: lanes a, b, g and h, 1 μ g; lane c, 0.1 μ g; lane d, 0.25 μ g; lane e, 0.5 μ g; lane f, 0.75 μ g. The assay conditions for lanes a–g were preincubation of lipocortin with phospholipase A_2 before initiation of the reaction by the addition of the substrate, except for lane a, where the *E. coli* membranes were omitted; and for lane b, in which Ca^{2+} was replaced with 10 mM-EGTA. The assay conditions for lane h were preincubation of the inhibitory protein (1 μ g) with the substrate before initiation of the reaction with the enzyme. The supernatants of each sample were analysed for phospholipase A_2 -inhibitory activity (each datum, expressed as percentage inhibition, is the average of triplicate determinations) and the pellets were analysed by SDS/polyacrylamide-gel electrophoresis followed by Coomassie Blue staining. Migration of the 32 kDa protein is indicated by an arrow.

Fig. 3. Binding of the 32 kDa protein to immobilized phospholipids

Incubations with phosphatidylserine (a) and phosphatidylinositol (b) were carried out in the presence of Ca^{2+} (lanes 1), after addition of an excess of EGTA (lanes 2) and in the presence of EGTA (lanes 3). The supernatants (S) and pellets (P) were analysed by SDS/polyacrylamide-gel electrophoresis.

ability of lipocortin to bind to immobilized phospholipids. Results in Fig. 3 show that the protein is able to bind reversibly to immobilized phospholipids, phosphatidylserine (Fig. 3a) and phosphatidylinositol (Fig. 3b), in a Ca^{2+} -dependent manner. The binding can be reversed by the addition of an excess of EGTA and is prevented in the presence of the chelating agent. These properties are consistent with those reported for the other proteins termed annexins, reinforcing the idea that the activity (inhibition of phospholipase A_2) is dependent upon binding to negatively charged phospholipids in the presence of Ca^{2+} (Davidson *et al.*, 1987; Haigler *et al.*, 1987; Aarsman *et al.*, 1987).

In order to characterize further the 32 kDa lipocortin from peripheral-blood mononuclear cells, we obtained amino acid sequence data. The *N*-terminus of the protein proved to be blocked, similarly to lipocortin I, calpactin I and protein II (Pepinsky *et al.*, 1986; Saris *et al.*, 1986; Weber *et al.*, 1987). The protein was therefore digested with trypsin after *S*-carboxymethylation. The peptides were separated by h.p.l.c. (Fig. 4) and sequenced (Fig. 5).

Fig. 4. Separation of tryptic peptides from the 32 kDa protein by h.p.l.c.

Peaks marked with an asterisk were pooled and rechromatographed on the same column with a gradient from 0 to 50% solvent B (results not shown).

The order of 253 amino acid residues was established in stretches ranging from 3 to 22 residues. The evidence thus obtained was sufficient to show a significant degree of homology with lipocortins I and II (Fig. 5) and pig protein II. The consensus sequences in particular were easily recognizable.

While this work was in progress, the cDNA sequence of a human placental inhibitor of blood coagulation named IBC [inhibitor of blood coagulation (Iwasaki *et al.*, 1987)] and PAP [placental anticoagulant protein (Funakoshi *et al.*, 1987a,b)] was published. Our data correspond exactly to the deduced amino acid sequence of the 317-residue inhibitor. Apart from several small hydrophilic peptides, which were probably present in the breakthrough peak, we were unable to locate one hydrophobic peptide and another larger hydrophilic one, corresponding together to positions 149–177.

In view of these results, we tested the anticoagulant activity of this protein in a biological assay by measuring activated partial thromboplastin time. Table 1 shows that this protein is also an inhibitor of intrinsic blood coagulation. To characterize further the mechanism of inhibition, we tested the ability of normal plasma containing 100 μg of inhibitor/ml to restore the coagulation of plasma depleted in intrinsic coagulation Factors VIII, IX, XI and XII. The results in Table 2 indicate that all the coagulation factors are 100% active, showing that lipocortin does not inhibit these factors directly but behaves rather like an 'antiphospholipid' (Thiagarajan *et al.*, 1980).

DISCUSSION

In this paper we present further biochemical and biological information about the 32 kDa lipocortin isolated from human mononuclear cells. The following findings indicate that it belongs to the new family of Ca^{2+} /lipid-binding proteins (Geisow *et al.*, 1987): (i) its purification is based on the ability of the protein to bind reversibly to biological membranes in a Ca^{2+} -dependent manner; (ii) it inhibits phospholipases A_2 from various sources (Rothhut *et al.*, 1987); (iii) binding studies confirm the reversible Ca^{2+} -dependent interaction with phospholipids (^3H)oleic acid-labelled *E. coli* membranes, immobilized phosphatidylserine and phosphatidyl-

The alignment shown is that proposed by Weber *et al.* (1987) for human lipocortins I (p35) and II (p36) and pig protein II. The sequences of lipocortins I and II and the inhibitor of blood coagulation (IBC) are taken from Huang *et al.* (1986), Kristensen *et al.* (1986) and Iwasaki *et al.* (1987), and Funakoshi *et al.* (1987b) respectively. The upper line indicates the partial sequences determined in the present work. Peptides are numbered according to their elution position from h.p.l.c. The b series peptides were obtained upon rechromatography of poorly resolved peptides (see legend to Fig. 3). Sequences carrying the same peptide number were obtained from a single chromatographic peak; they could easily be distinguished from one another because of different peptide amounts (e.g. T66 and T21). Lower-case letters indicate tentative identification. In addition to the peptides shown here, which were sequenced without prior knowledge of the blood coagulation inhibitor sequence, 15 more residues could be assigned in mixtures with knowledge of the published sequence. No peptide was found with a sequence that did not correspond to the published one.

Activated partial thromboplastin time (APTT) was determined as indicated in the text.

Concn. of protein in plasma ($\mu\text{g/ml}$)	APTT (s)
0	41
36	50
100	190
160	290

inositol); (iv) protein sequencing results show a significant degree of homology with lipocortins I and II (Huang *et al.*, 1986; Kristensen *et al.*, 1986) and pig protein II (Weber *et al.*, 1987); furthermore over the known portion of the sequence (80%) they reveal complete identity with the sequence of the recently described inhibitors of blood coagulation (Iwasaki *et al.*, 1987; Funakoshi *et al.*, 1987b), with the partial sequence of endonexin II (Schlaepfer *et al.*, 1987; Haigler *et al.*, 1989)

Lipocortins are thought to inhibit phospholipases A₂ by binding to the negatively charged phospholipid substrate in the presence of Ca²⁺. This mechanism may also be involved in the anticoagulant properties of the protein, since binding of coagulation factors on phosphatidylserine is essential for initiation of the process. These inhibitions may implicate a competition between lipocortin and either phospholipase A₂ or the coagulation factors for binding to phospholipids. The 32 kDa protein binds to phosphatidylinositol in a Ca²⁺-dependent manner. Since this phospholipid is a substrate of phospholipase C, it would be of interest to test whether the protein has an inhibitory effect on the enzyme activity. Lipocortins are regulated by various kinases affecting their binding affinity for the phospholipids (Sheets *et al.*, 1987; Powell & Glenney, 1987). We have shown (Coméra *et al.*, 1989) that the 32 kDa lipocortin is not phosphorylated by the epidermal-growth-factor-dependent tyrosine kinase and by protein kinase C *in vitro*.

The presence of the 32 kDa lipocortin in various cells involved in the inflammatory (monocytes, lymphocytes)

Table 2. Assays of coagulation Factors VIII, IX, XI and XII in normal plasma containing 100 µg of lipocortin/ml (final concentration) or not

Activated partial thromboplastin time (APTT) was determined as indicated in the text.

Coagulation factor	Dilution	APTT (s)	
		Normal plasma + saline	Normal plasma + 100 µg of 32 kDa protein/ml
VIII	1/10	54	55
	1/20	60	61
	1/40	67	68
IX	1/10	48	48
	1/20	53	52
	1/40	58	58
XI	1/10	49	49
	1/20	56	57
	1/40	66	65
XII	1/10	60	62
	1/20	69	70
	1/40	79	82

and coagulation processes (platelets, endothelial cells) reinforces the idea of a possible 'patho'-physiological role of the protein. Indeed, in the coagulation process one might expect endothelial cell and platelet damage and consequently protein extrusion. Both types of cells are targets for binding of coagulation Factors IX and X on their surface in a Ca^{2+} -dependent manner (Heimark & Schwartz, 1983; Stern *et al.*, 1983). Therefore the protein might compete with these factors for the surface phospholipids. An extracellular role for the 32 kDa protein in the inflammatory process is more questionable, since only lipocortin I has been described as a glucocorticoid-induced extracellular protein without any leader peptide. But the relationship with glucocorticoids has not yet been investigated for the 32 kDa protein. A possible association of the protein with exocytosis granules could be proposed, since such a phenomenon has been reported for the members of this family in chromaffin granules (Creutz, 1981). Nevertheless its function as an extracellular mediator involved in inflammation and coagulation has not yet been demonstrated.

In conclusion, despite controversy in the proposed nomenclature for these different factors, a consensus is emerging from the different studies concerning some properties of this protein. It is a protein present in many cell types, except in human erythrocytes and neutrophils. It binds to phospholipids in a Ca^{2+} -dependent manner, properties that may explain phospholipase A_2 -inhibitory activity. The anticoagulant activity of the protein was also in part attributed to its Ca^{2+} -dependent phospholipid-binding properties (Iwasaki *et al.*, 1987; Funakoshi *et al.*, 1987*a, b*) and was recently described for some other members of the family (Chap *et al.*, 1988). Inhibition of human Factor VIIa-tissue factor activity by placental anticoagulant protein has been described (Kondo *et al.*, 1987), but the mechanism of inhibition was shown to involve binding to the phospholipid portion of the tissue-factor lipoprotein. Nevertheless the physiological role of the intracellular protein is still poorly understood. One can propose a role for the 32 kDa

lipocortin as an intracellular link between membrane stimulation and cellular response, or as a regulator of cytoskeleton-membrane interactions, as has been proposed for other members of this family of proteins.

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