Biochemistry

© Copyright 1988 by the American Chemical Society

Volume 27, Number 18 September 6, 1988

Perspectives in Biochemistry

Ca^{2+}-Dependent Phospholipid- (and Membrane-) Binding Proteins

Claude B. Klee

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Received April 5, 1988

The best known family of Ca^{2+}-regulated proteins is the "EF-hand" group, which includes parvalbumin, calmodulin, troponins, and other small, soluble proteins (Kretsinger, 1980). These proteins bind Ca^{2+} with high affinity and, in some cases, subsequently activate enzymes or contractile proteins. In recent years, a second group of Ca^{2+}-binding proteins has come to be recognized. These are widely distributed, phospholipid- and membrane-binding proteins. They may be important in the control of cell proliferation through their modulation of arachidonic acid metabolism and because they are substrates of growth factor receptor kinases.

These proteins were first identified, and isolated, by their Ca^{2+}-dependent binding to cellular membranes and their abilities to promote membrane aggregation [for reviews, see Geisow and Walker (1986) and Glenney (1987)]. The Ca^{2+}-dependent binding to membranes of some of these proteins is resistant to treatment with nonionic detergents, suggesting that they may interact not only with membranes but also with cytoskeleton proteins. Because of their Ca^{2+}-dependent interaction with phospholipids and actin, two of the proteins were named calpactins (Glenney, 1986a). The Ca^{2+}-dependent membrane binding of calpactins and their association with the cytoskeleton suggested that they may be involved in the control of secretion and cell motility. Recent evidence indicates that calpactins may play a more general role in cellular regulation. For example, calpactins are substrates of retroviral and growth factor receptor kinases and, thus, are presumably involved in the regulation of cell growth [reviewed by Cooper and Hunter (1985)]. The calpactins are now known to be identical with lipocortins, inhibitors of phospholipase A_2 whose synthesis is induced by steroid hormones. Lipocortins are presumed to be responsible for the antiinflammatory effect of the steroids [reviewed by Flower et al. (1984) and Hirata (1984)].

Nomenclature. The major Ca^{2+}-dependent membrane-binding proteins that have been described so far are listed in Table I. They were originally isolated from a variety of tissues and were given names related to their source or properties. Thus, several of the proteins in this family were given a number of different names. In Table I and the rest of this review, a single name has been chosen for each group of similar proteins. Synonyms are provided for cross-referencing purposes. With some exceptions, the molecular weight of most of these proteins falls within two groups, one near 70 000 (the proteins belonging to this group are referred to as p70) and the other between 32 000 and 39 000. The latter group contains two related proteins, calpactin I and calpactin II, which have been extensively studied. Calpactin I (originally called protein I) was first isolated from porcine intestinal epithelial cells (Gerke & Weber, 1984) and later purified in good yields from bovine lung (Glenney et al., 1987; Khanna et al., 1987) and human placenta (Huang et al., 1986; Haigler et al., 1987; Glenney et al., 1987, Hayashi et al., 1987). A similar protein was found in smooth muscle membranes (Raeymakers et al., 1984) and mouse mammary epithelial cells (Braslau et al., 1984). Calpactin II was isolated from bovine lung and human placenta (Glenney & Tack, 1985; Glenney, 1986b; Huang et al., 1986; Khanna et al., 1987; Haigler et al., 1987; Glenney et al., 1987). Calelectrin was purified from the electric organ of the electric fish *Torpedo marmorata* (Walker, 1982). Chromobindins and synexins were identified in bovine adrenal medulla chromaffin cells (Creutz et al., 1978, 1983; Odenwald & Morris, 1984) and endonexin in liver and adrenal medulla (Geisow et al., 1984). Two Ca^{2+}-dependent membrane-binding proteins (p33 and p28) in human lymphocytes are related to calpactins and endonexin, respectively (Owens & Crumpton, 1984). Several proteins, with similar Ca^{2+}- and phospholipid-binding properties with M_r around 70 000, have been described in lymphocytes, liver, placenta, lung, and adrenal medulla (Table I). Five related proteins (M_r 67 000, 35 000, 33 000, and 30 000), called calcimedins, were purified from smooth muscle by Dedman and Moore (1982) using the Ca^{2+}-dependent affinity chromatography on phenothiazine columns developed for the purification of calmodulin. The identification of calcimedins as Ca^{2+}-dependent phospholipid-binding proteins was suggested by their molecular weight and Ca^{2+}-dependent affinity for the hydrophobic support and later confirmed by immunological studies (Smith & Dedman, 1986). The Ca^{2+}-dependent binding of calelectrins to phenyl-Sepharose was also observed by Sudhof et al. (1984). The

This article not subject to U.S. Copyright. Published 1988 by the American Chemical Society

Table I: Ca^{2+}-Dependent Membrane-Binding Proteins

name	synonyms	subunit $M_r \times 10^{-3}$	phospholipid[a] specificity	pI	references[b]
calpactin I		38	PS, PI	7.5	1–8
	p36 (substrate $pp60^{src}$)	+			
	lipocortin II	11			
	chromobindin 8				
	lymphocyte p33				
calpactin II		39	PS	6.8	3–6, 9–11
	p35 (substrate EGF receptor)				
	lipocortin I				
	chromobindin 9				
endonexin		32.5	PI, PE, PA	5.6	1, 12, 13
	protein II				
	lymphocyte, p28				
calcimedins		30–33			14
		35			
		67			
synexin I		47	PS		15
synexin II		56	PS		16
calelectrin		35–36	PS	5.6	21
p70		67–73	PI, PA, PE	5.9	1, 12, 13, 17–20
	protein III				
	chromobindin 20				
	synhibin				

[a] PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid. [b] (1) Geisow & Walker, 1987; (2) Gerke & Weber, 1984, 1985a; (3) Glenney, 1985; Glenney et al., 1987; (4) Huang et al., 1986; (5) Hayashi et al., 1986; (6) Khanna et al., 1987; (7) Erickson et al., 1984; (8) Hexham et al., 1986; (9) Fava & Cohen, 1984; (10) Wallner et al., 1986; (11) Haigler et al., 1987; (12) Geisow et al., 1984, 1986; (13) Shadle et al., 1985; (14) Moore & Dedman, 1982; (15) Odenwald & Morris, 1983; (16) Creutz et al., 1978, 1983; (17) Owens & Crumpton, 1984; (18) Owens et al., 1984; (19) Fauvel et al., 1987; (20) Davidson et al., 1987; (21) Sudhof et al., 1985.

inhibitory effect of phenothiazine on the interaction of such proteins with phospholipids (Shadle & Weber, 1987) is further evidence for the lack of specificity of phenothiazines for calmodulin.

Because of their wide distribution, detailed characterization, and homology with other Ca^{2+}-dependent phospholipid-binding proteins, the calpactins are the focus of this review. Since calpactins have been shown to be immunologically and structurally related to many other Ca^{2+}-dependent membrane-binding proteins, the analogy between calpactins and other members of this family of proteins will be discussed whenever possible.

General Properties. Calpactins are proteins that remain associated with membranes in the presence of Ca^{2+} and nonionic detergents and are released by EGTA. They bind actin and fodrin (the spectrin analogue of nonerythroid cells) (Gerke & Weber, 1984, 1985a; Glenney, 1986b). Their M_r values on SDS gels vary between 34 000 and 39 000. They can be purified, to about 50% homogeneity, from membrane cytoskeletons by successive cycles of Ca^{2+} precipitation and EGTA solubilization (Gerke & Weber, 1984; Glenney, 1986b; Glenney et al., 1987). Calpactin I is a tetramer composed of two M_r 36 000 heavy chains and two M_r 11 000 light chains (Gerke & Weber, 1985a; Glenney & Tack, 1985). Calpactin II, more acidic and with a slightly larger molecular weight, is usually isolated as a monomeric heavy chain (Glenney & Tack, 1985; Glenney, 1986b; De et al., 1986).

In the presence of micromolar Ca^{2+}, these proteins specifically bind acidic phospholipids, usually those primarily found at the cytoplasmic face of the membranes. Calpactins and synexins preferentially bind phosphatidylserine (Gerke & Weber, 1984, 1985a; Glenney, 1986a; Odenwald & Morris, 1983; Creutz et al., 1983; Johnsson et al., 1986a). Other Ca^{2+}-dependent membrane-binding proteins exhibit a different specificity. Endonexin and p70 interact selectively with phosphatidylinositol, phosphatidylethanolamine, and phosphatidic acid (Geisow et al., 1986).

The Ca^{2+} dependence of these interactions was taken as evidence that calpactins are Ca^{2+}-binding proteins. Ca^{2+} binding was subsequently demonstrated, indirectly by Ca^{2+}-induced conformational changes (Gerke & Weber, 1985a; Johnsson et al., 1986a; Schlaepfer & Haigler, 1987) and directly by the method of Hummel and Dreyer or by equilibrium dialysis (Gerke & Weber, 1985a; Shadle et al., 1985; Glenney, 1986a; Glenney et al., 1987). The low affinity of the purified proteins for Ca^{2+}, in both the presence and absence of Mg^{2+} [$K_d = 10^{-4}$ M for calpactin I (Gerke & Weber, 1984, 1985a; Glenney, 1985), 2×10^{-4} M for synexin I (Creutz et al., 1978), 2.5×10^{-5} M for protein II (Shadle et al., 1985), and 10^{-5}–10^{-4} M for calelectrin (Sudhof et al., 1985)], has hindered the precise determination of the number of binding sites and the binding constants. The high molecular weight proteins isolated from lymphocytes (p68) or liver (protein III) are the only members of this family found to have, in the absence of phospholipid, a single high-affinity Ca^{2+}-binding site (in the micromolar range) (Owens & Crumpton, 1984; Shadle et al., 1985).

As expected from the fact that phospholipid binding requires Ca^{2+}, addition of the specific phospholipids known to interact with these proteins greatly increases the affinity for Ca^{2+} (Sudhof et al., 1984; Glenney, 1986a; Geisow et al., 1986; Johnsson et al., 1986a; Glenney et al., 1987; Schlaepfer & Haigler, 1987; Shadle & Weber, 1987). In the presence of phosphatidylserine (but not phosphatidylcholine, which does not bind to calpactins) calpactin I binds 2 mol of Ca^{2+}/mol with a K_d of 4.5×10^{-6} M (Glenney, 1986a). Under the same conditions, the binding of 2 mol of Ca^{2+} to calpactin II exhibits positive cooperativity and an apparent K_d of 10^{-5} M (Glenney et al., 1987). These dissociation constants are in good agreement with the concentration of Ca^{2+} (10^{-5} M) needed to support 50% maximal binding of calpactins to liposomes in the presence of millimolar Mg^{2+} and at physiological ionic strength (Gerke & Weber, 1985a; Glenney et al., 1987). A similar enhancement of affinity for Ca^{2+} in the presence of cytoskeletal and membrane proteins is likely but has not been reported.

The Ca^{2+}-dependent interaction of several ligands provides a mechanism for increasing the low affinity of this class of proteins for Ca^{2+}. It may also result in cooperative binding of Ca^{2+}, thus allowing the proteins to respond to small changes

in intracellular Ca^{2+} concentration. A similar mechanism, described in detail by Huang et al. (1981), applies to the highly cooperative, Ca^{2+} stimulation of calmodulin-regulated enzymes first observed with cAMP phosphodiesterase (Crouch & Klee, 1980). Rigorous demonstration of this mechanism would require detailed measurements of the effects of ligand concentration on Ca^{2+} affinity.

Relationship between Calpactins, the Substrates of Tyrosine Kinases, and Lipocortins. The substrate of $pp60^{src}$ (339 residues) is the large subunit of calpactin I and is identical with lipocortin II. The substrate of the EGF receptor kinase (346 residues) is the same as calpactin II or lipocortin I. The chemical and immunological similarity between the heavy chain of calpactin I and p36, the substrate of Rous sarcoma virus tyrosine kinase ($pp60^{src}$), was demonstrated by Gerke and Weber (1984). At the same time p36 was shown to be associated with a polypeptide of M_r 6000 (Erikson et al., 1984). The M_r 36000 Ca^{2+}-dependent binding protein of chromaffin granules was also identified as p36, supporting Gerke and Weber's conclusions (Geisow et al., 1984). Definitive identification of calpactin I as the substrate of $pp60^{src}$ followed the cloning and sequence determination of cDNAs for calpactin I heavy chains from transformed NIH 3T3 cells (Saris et al., 1986) and from Madin–Darby bovine kidney cells (Kristensen et al., 1986). The positions of 173 residues of p36, determined by protein sequencing (Kristensen et al., 1986), coincided with those of the same residues in the predicted sequence of bovine kidney calpactin I. The bovine protein shows 98% identity with the predicted structure of its murine and human equivalents (Huang et al., 1986; Kristensen et al., 1986).

The similarity of the substrate of the EGF receptor tyrosine kinase to the Ca^{2+}-dependent membrane-binding proteins was noticed by Fava and Cohen (1984). This substrate, routinely called p35, exhibits Ca^{2+}-dependent binding to membranes and is biochemically and immunologically related to but not identical with p36. Although p35 is phosphorylated by $pp60^{src}$, the EGF receptor does not phosphorylate p36 (Fava & Cohen, 1984). In contrast to p36, which is predominantly found as an oligomer, p35 is monomeric (Glenney, 1986b; De et al., 1986) and was tentatively identified as calpactin II (Glenney, 1986b).

The discovery that the substrates of the tyrosine kinases are members of the lipocortin gene family marked another major advance in the characterization of calpactins. Lipocortins, first described as inhibitors of phospholipase A_2, are present in many cells and were shown to be phosphorylated by tyrosine as well as serine and threonine kinases (Hirata et al., 1984). It was proposed that the inhibitory activity of lipomodulin, a 40-kDa form of lipocortin, is regulated by tyrosine phosphorylation (Hirata, 1984). Determination of the partial amino acid sequence of rat lipocortin revealed its similarity to the substrate of the EGF receptor kinase, p35 (Pepinsky et al., 1986). Wallner et al. (1986) cloned the human lipocortin cDNA using oligonucleotides derived from the DNA sequence of rat lipocortin and expressed it in *Escherichia coli*. The protein, synthesized in bacteria, inhibits phospholipase A_2 as does native lipocortin and, like p35, is phosphorylated by the EGF receptor near its amino terminus. In rat peritoneal cells, the level of a mRNA that hybridizes with the lipocortin gene is increased following treatment with steroids (Wallner et al., 1986). Comparison of the primary structures of bovine and murine calpactin I heavy chains with that of human placenta lipocortin revealed 50% identity between calpactin I and lipocortin.

The structural relationships between calpactin I, calpactin II, and lipocortin were further clarified by Huang et al. (1986), who purified two 35-kDa inhibitors of phospholipase A_2, lipocortin I and lipocortin II, from human placenta. Lipocortin II was shown to be the human homologue of p36, the substrate of $pp60^{src}$, and the heavy chain of calpactin I from intestinal epithelium cells. Like calpactin I, lipocortin II has been isolated as an 85-kDa oligomer called lipocortin 85 (Khanna et al., 1987). Peptide mapping, sequence, and immunological analysis of lipocortin I confirmed its identification as p35, the substrate of EGF receptor kinase isolated by Fava and Cohen (1984).

Lipocortin I has now been identified as calpactin II. Glenney et al. (1987) determined the amino-terminal sequence of a low molecular weight variant of bovine calpactin II, which has an unblocked amino terminus (probably as the result of adventitious proteolysis). With the exception of two amino acids, the 28-residue amino-terminal sequence was identical with that of residues 12–39 of human lipocortin I. One-fourth of the sequence of p35 is identical with the sequence of human lipocortin I (Haigler et al., 1987). Phosphorylation of calpactin II by the EGF receptor provided further evidence for this identification.

The substrates of the tyrosine kinases are therefore members of the family of Ca^{2+}-dependent membrane-binding proteins as had been predicted by Fava and Cohen (1984). This identification is consistent with the effects of Ca^{2+} and phospholipids on the phosphorylation of the two substrates by their respective kinases (Fava & Cohen, 1984; Glenney, 1985; De, 1986; Glenney et al., 1987) or by protein kinase C (Gould et al., 1984). In agreement with the relatively low affinity of calpactins for Ca^{2+}, the stimulation of the phosphorylation of p35 (Fava & Cohen, 1948) and of p36 (Glenney, 1985) requires high Ca^{2+} concentrations. Phospholipids, which increase the affinity of calpactins for Ca^{2+}, decrease the concentration of Ca^{2+} needed to support 50% phosphorylation of calpactin I with $pp60^{src}$ and of p35 with EGF receptor from 10^{-4} M to less than 10^{-5} M (Glenney, 1985; De et al., 1986). The demonstration that the substrates of the two tyrosine kinases are distinct proteins provides a basis for their different biological activities. Perhaps the substrates of other retroviral or growth factor kinases are also Ca^{2+}-dependent membrane-binding proteins, some of which may already have been isolated.

Insulin receptor kinase substrates may belong to a different family of proteins. Ca^{2+} has no effect on the phosphorylation of two proteins (M_r 50000 and 35000) that are believed to be endogenous substrates of the insulin receptor kinase in liver cells. These proteins are not phosphorylated by the EGF receptor nor is p35 phosphorylated by the insulin receptor (Kwok & Yip, 1987).

The Small Subunit of Calpactin I Is a Homologue of S-100α. The heavy chain of calpactin I harbors the binding sites for Ca^{2+}, phospholipid, and cytoskeletal proteins. Ca^{2+} binding to calpactin I or to its isolated 36-kDa subunit induces conformational changes, which affect the environment of at least two tyrosines and the single tryptophan, and favors interaction with actin and fodrin (Gerke & Weber, 1985a; Glenney, 1986a). Although smaller spectroscopic changes are observed when the isolated large subunit is studied, the presence of the small subunit is not an absoulte requirement. The conformational of the small subunit is apparently not affected by Ca^{2+}, and it binds neither actin nor fodrin (Gerke & Weber, 1985a; Glenney, 1986a).

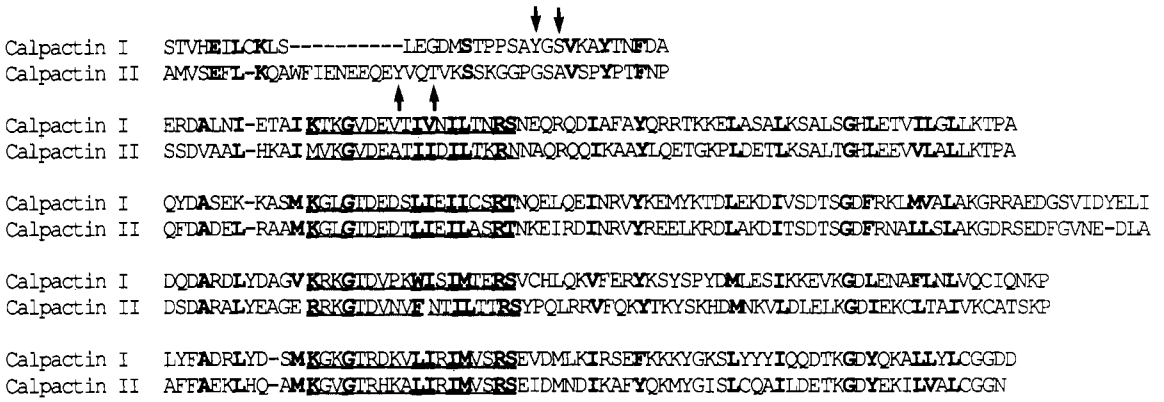

FIGURE 1: Comparison of the predicted amino acid sequences of bovine calpactin I and human calpactin II. The first two lines show the amino-terminal tails of the two proteins. The sites of phosphorylation by pp60^{src}, EGF receptor, and protein kinase C are indicated by the arrows. The four repeat units of the two proteins are shown in the next eight lines allowing the best alignment of the conserved residues in each repeat. Amino acids that are identical or conservatively replaced (Ser for Thr, Glu for Asp, Arg for Lys, and hydrophobic residues) in at least seven repeats are shown as bold characters. The consensus sequence of Geisow et al. (1986) is underlined.

Amino acid sequence determination of the small subunit (M_r 11 000) revealed a similarity to S-100α, a Ca^{2+}-binding member of the EF-hand family (Gerke & Weber, 1985b; Glenney & Tack, 1985). Allowing a three amino acid deletion, 43 of the 91 residues of the calpactin light chain occupy the same position as the corresponding amino acids in S-100α (Isobe & Okuyama, 1981). Like S-100α, the light chain of calpactin I has a tendency to dimerize; however, it does not bind Ca^{2+} (Gerke & Weber, 1985a; Johnsson et al., 1986a). The lack of Ca^{2+} binding to the calpactin light chain could be due to a deletion in the first Ca^{2+} site and incompatible amino acid replacements (Cys for Asp, Arg for Gly, Lys for Glu, and Ser for Glu) in the second. Alternatively, the lack of Ca^{2+} binding may be due to the presence of nonexchangeable Ca^{2+} or Zn^{2+} (Gerke & Weber, 1985b). The small subunit of lymphocyte calpactin I also does not bind Ca^{2+} and is homologous to S-100α (Hexham et al., 1986).

Structure–Function Relationships. As noted by Saris et al. (1986), calpactin I (large subunit, p36) consists of a core of four repeating units and an amino-terminal tail about 30 residues long. Similar repeating units, also present in calpactin II, may be responsible for the immuno-cross-reactivity between the two proteins (Khanna et al., 1987). Alignment of the sequences of calpactin I and calpactin II (Figure 1) shows 30% identity between any two of the four repeats. Each of the repeats also contains a 17-residue structure from which a consensus sequence, called the endonexinn fold, was derived by Geisow et al. (1986) (Figure 2). The endonexin fold is a unit shared by at least four Ca^{2+}-dependent membrane-binding proteins (endonexin, calelectrin, calpactin I, and calpactin II). Much less similarity is observed between the 34- and 42-residue amino-terminal tails of calpactin I and II. The primary structure of protein II from intestinal cells (Weber et al., 1987) and that of p68 from lymphocyte (Crompton et al., 1988) confirm the generality of these observations. They too are composed of a core of repeat units, four for protein II and eight for p68, each of which contains the endonexin fold illustrated in Figure 2.

These structural characteristics match the organization of the functional domains of calpactins revealed by limited proteolysis. Controlled digestion with chymotrypsin removes a portion of the blocked amino terminus of calpactin I to yield a M_r 33 000 fragment and leaves the light chain intact (Glenney & Tack, 1985; Johnsson et al., 1986a). The M_r 33 000 fragment preserves the ability to undergo a Ca^{2+}-dependent conformational change, and binds actin and phospholipids, but loses the ability to interact with the dimer formed

CALELECTRIN	G	F	G	Σ	D	E	D	V	Φ	Φ	D	Φ	Φ	T	Q	R
	-	A	-	-	S	-	N	-	-	-	E	-	-	A	S	-
CALPACTIN I	T	K	-	V	-	-	V	T	-	-	N	-	-	-	N	- Σ
	-	L	-	-	-	-	-	S	-	-	E	-	-	C	S	- -
	R	K	-	-	-	V	P	K	-	-	S	-	-	-	E	- -
	-	K	-	-	R	D	K	-	-	-	R	-	-	V	S	- -
CALPACTIN II	V	K	-	V	-	-	A	T	-	-	-	-	-	-	K	- N
	-	L	-	-	-	-	-	T	-	-	E	-	-	A	S	- -
	R	K	-	-	-	V	N	-	-	N	T	-	-	-	T	- -
	-	V	-	-	R	H	K	A	-	-	R	-	-	V	S	- -
PROTEIN II	-	L	-	-	-	-	-	A	-	-	S	-	-	A	Y	- -
	-	A	-	-	-	-	G	C	-	-	E	-	-	A	S	- -
	K	W	-	-	-	-	V	K	-	-	T	-	-	C	S	- N
	-	L	-	-	-	D	N	T	-	-	R	-	-	V	S	- A
P68	-	F	-	-	-	K	E	A	-	-	-	-	-	-	S	- -
	-	I	-	-	-	-	K	C	-	-	E	-	-	A	S	- -
	K	W	-	-	-	-	A	Q	-	-	Y	-	-	G	N	- -
	-	L	-	-	R	D	N	T	-	-	R	-	-	V	S	- -
	-	L	-	-	-	-	-	T	-	-	-	-	-	-	H	- -
	-	A	-	-	-	-	K	A	-	-	E	-	-	A	T	- -
	D	K	-	-	L	-	T	R	-	-	T	-	-	C	T	- -
	-	A	-	-	-	-	K	T	-	T	R	-	-	V	S	- -

FIGURE 2: Consensus sequences of Ca^{2+}-dependent membrane-binding proteins. The 17-residue sequences, identified by Geisow et al. (1986) as a common feature of four Ca^{2+}-dependent membrane-binding proteins and detected in the repeat units of calpactin I, calpactin II, protein II, and p68, are aligned. Identical residues are indicated by a dash; Φ, hydrophobic residues, Val, Ile, Leu, Met, Tyr, and Phe; Σ, Ser or Thr.

by the two small subunits and is therefore monomeric (Glenney, 1986a; Glenney et al., 1986; Johnsson et al., 1986a,b). The phosphorylatable residues Tyr-23, Tyr-20, Ser-25, and Thr-23 (the last two phosphorylated by protein kinase C) located in the amino-terminal tails (Glenney & Tack, 1985; Gould et al., 1986; Pepinsky & Sinclair, 1986; Weber et al., 1987; De et al., 1986) are removed upon proteolysis of calpactin I (Johnsson et al., 1986b) and calpactin II (Glenney et al., 1986; De et al., 1987; Schlaepfer & Haigler, 1987). A major difference between the two sequences is a deletion, in calpactin I, of the stretch of glutamic acids preceding Tyr-20, the recognition site for the EGF receptor kinase (De et al., 1986) (Figure 1). Accordingly, the EGF receptor kinase does not phosphorylate calpactin I (Fava & Cohen, 1984). Protein II with the shortest protease-sensitive amino-terminal tail lacks the tyrosyl residue phosphorylated by tyrosine kinases and is probably too short to contain the binding site for a small subunit, but contains a threonine residue at position 6, which is a likely candidate for the site

of phosphorylation observed with protein kinase C (Johnsson et al., 1986b; Weber et al., 1987).

Although the structurally conserved core of repeat units in the two proteins is associated with binding of Ca^{2+}, phospholipids, and cytoskeletal proteins, identification of the binding sites for these ligands has not yet been achieved. Analysis of the amino acid sequences of the two calpactins fails to reveal a sequence homologous to that of the Ca^{2+}-binding loops of the EF-hand Ca^{2+}-binding proteins (Kretsinger & Creutz, 1986). The predicted secondary structure of lipocortin I suggests a high α-helix content. Assuming two deletions in the Ca^{2+}-binding loop, the hydrophobic residues along the helices can be aligned with the hydrophobic residues of the vitamin D dependent Ca^{2+}-binding protein calbindin, an EF-hand Ca^{2+}-binding protein (Szebenyi & Moffat, 1986). Geisow et al. (1986) modeled the first and second helices of the second repeat of lipocortin I to the coordinates of calbindin and predicted a novel Ca^{2+} site providing octahedral coordination for the calcium ion and accessibility to phospholipids. Since lipocortin I contains four repeats and only two Ca^{2+} sites and the lymphocyte protein p68 contains eight repeats and only one Ca^{2+} site, modeling of the other repeats may yield additional information on the structural requirements for this class of sites.

Relationships of Calpactins to Other Ca^{2+}-Binding Proteins of the Same Family. The sequence data show that calpactin I and calpactin II, endonexin (protein II), and lymphocyte p68 share structural features involved in Ca^{2+} and phospholipid binding but differ in their amino termini. Three related types of protein can be distinguished with the available antibodies. One group includes calpactins I and II. The members of this group react with an antibody to chick fibroblast p36; they include calpactin I, the 33-kDa polypeptide of human lymphocytes (Davies & Crumpton, 1985), and the 36-kDa chromobindin (Geisow et al., 1984). The same antibody reacts weakly with calpactin II, which is better detected with specific antibodies against p35 from A431 cells. Anti-calpactin II antibody also reacts with a 65-kDa protein of human placenta (De et al., 1986). None of the other high molecular weight proteins, grouped as p70, are recognized by anti-calpactin antibodies. A second group of proteins is identified by anti-endonexin antibodies. This group consists of the antigen itself, p32.5 (endonexin from adrenal medulla), protein II from intestinal cells, and lymphocyte p28 (Sudhof et al., 1984; Davies & Crumpton, 1984). Anti-endonexin also recognizes p67 of pig lung (Fauvel et al., 1987).

Structural differences between calpactins and endonexins may reflect functional differences. Calpactins bind not only phospholipids but also actin and fodrin. In contrast, association of endonexin, protein II, and most of the high molecular weight proteins (p70) with the cytoskeleton has not been clearly established (Geisow et al., 1984; Weber et al., 1987). Endonexin and protein II are not phosphorylated by $pp60^{src}$ (Geisow et al., 1986; Weber et al., 1987), and lung p70 is a poor substrate for protein kinase C (Fauvel et al., 1987).

The third group of Ca^{2+}-binding proteins, not as well characterized, includes calcimedins and synexins. These are immunologically distinguishable from those already discussed (Geisow & Walker, 1986). Anti-calcimedin antibodies do not recognize calpactins, bind adrenal medulla p32.5, p35, p67, and calelectrin, and also cross-react with synexin (Smith & Dedman, 1986). Determination of the primary structure of this group of proteins is needed to assess the generality of the repeat structure revealed by the sequence analyses of the calpactins, endonexin, and p68 protein and would help to define the criteria for identification of this class of Ca^{2+}-binding proteins.

The list of proteins in Table I is far from complete. New phospholipid- and Ca^{2+}-binding proteins are being described regularly. An inhibitor of phospholipase A_2, immunologically different from calpactins I and II, is present in several rat tissues (Hayashi et al., 1987). Is this a degradation product of calpactin or a novel protein? Two M_r 67 000 proteins have been distinguished in lung on the basis of their phospholipid specificities and abilities to inhibit phospholipase A_2 (Fauvel et al., 1987).

Despite the reported absence of calpactin from brain, this organ contains larger amounts of Ca^{2+}-binding proteins than any other tissue. The functions and identities of many of these proteins remain to be established. One of these proteins (M_r 27 000) contains two specific high-affinity Ca^{2+}-binding sites (K_d = 0.2 μM) and is an inhibitor of phospholipase A_2. It is premature to identify this protein as a calpactin since it has not been tested for phospholipid binding or tyrosine phosphorylation (Tokuda et al., 1987a). Another protein from brain (M_r 48 000) is a tyrosine kinase substrate that binds Ca^{2+} ($K_d = 10^{-5}$ M) but does not inhibit phospholipase A_2 (Tokuda et al., 1987b). Brain also contains a Ca^{2+}-dependent membrane-binding protein, p68, which does not bind the antibody to liver p70 but is immunologically related to lymphocyte p68 and calcimedins (Rhoads et al., 1985). In thymus the molecular weight of calpactin II is different from that isolated from A431 cells (Fava & Piltch, 1987), suggesting that calpactins and related proteins may exist as tissue-specific isoforms. Definitive identification of these proteins is made difficult by their size and immunological heterogeneity, but their common characteristics, Ca^{2+} and phospholipid binding, seem to be associated with a particular sequence that is recognized by an anti-calelectrin antibody. This antibody, raised against denatured calelectrin, is less specific than antibodies raised against mammalian proteins and can be used to identify similar epitopes in different proteins.

The calelectrin antibody recognizes a M_r 36 000 chromobindin, p28 from lymphocytes, M_r 34 000 proteins in brain, liver, and intestinal cells, and brain p68 (Sudhof et al., 1984; Geisow et al., 1984; Rhoads et al., 1985). Geisow et al. (1986) isolated peptides from five different Ca^{2+}-dependent membrane-binding proteins that contain this epitope, the 17-residue "endonexin fold". As noted earlier, this consensus sequence has now been mapped in the four repeat units of calpactin I, lipocortin I, and protein II and in the eight repeat units of lymphocyte p68. Many or all Ca^{2+}-dependent membrane-binding proteins may be related to one another as shown by Western blot analysis with anti-calelectrin antibody of proteins resolved by two-dimensional electrophoresis (Glenney & Tack, 1985; Geisow & Walker, 1986). Identification of domains related to the individual properties of these proteins by specific antibodies and by phosphorylation with specific kinases will speed progress in this area. The interpretation of the immunological studies presently available is confused by the variable specificity of the antibody preparations used in different laboratories.

Tissue Distribution and Cellular Localization. The Ca^{2+}-dependent membrane-binding proteins coexist in a wide variety of murine, porcine, bovine, and human tissues (Geisow et al., 1984; Davies & Crumpton 1985; Shadle et al., 1985). Western blots of tissue homogenates with antibodies to calpactins I and II showed their uneven distribution (Glenney et al., 1987; Hayashi et al., 1987; Khanna et al., 1987; Pepinsky et al., 1986; Huang et al., 1986; De, 1986; Liu et al., 1987).

Calpactin I is the predominant form in most tissues. Lung and placenta are the richest sources (1% and 0.2% of total protein, respectively). Lower but significant levels are found in spleen, adrenal medulla, kidney, intestinal epithelium, smooth and cardiac muscle, ascitis tumor cells, and rat peritoneal exudates. Brain, skeletal muscle, liver, platelets, and erythrocytes contain very little if any calpactin I. The distribution of calpactin II is narrower. Only lung, placenta, spleen, and smooth muscle have been reported to contain significant amounts of calpactin II. The highest levels of the two forms of calpactin are found in epithelial cells. In thymus a protein cross-reacting with calpactin II is found in the keratinized epithelial cells and is absent from thymocytes (Fava & Pitch, 1987).

Calpactin I is detected by most cells in culture, even those derived from tissues that lack this protein. Lymphoid, epithelial PC12, neuroblastoma, glial, and macrophage cells all contain calpactin I. Calpactin II is absent from lymphoid and neuroblastoma cells. It has been detected only in glial, melanoma, and adrenocarcinoma cells, myoblasts, fibroblasts, and macrophages (Huang et al., 1986; Fava & Cohen, 1984).

The cellular localizations of calpactin I and calpactin II, determined by immunocytochemistry, are consistent with their biochemical properties. The heavy and light chains of calpactin I colocalize with the actin-binding proteins that form a reticular network underneath the plasma membrane (Cheng & Chen, 1981; Gerke & Weber, 1984; Glenney et al., 1987). In intestinal cells, calpactin I is found predominantly in the terminal web, and in fibroblasts and many cells in culture, it is associated with the plasma membrane (Gerke & Weber, 1985; Geisow et al., 1984). The localization of calpactin II is not as clearly defined; it is recovered in the soluble fraction after homogenization (Zokas & Glenney, 1987) but may be associated with membranes that differ from those binding calpactin I, perhaps those of intracellular organelles (Sawyer & Cohen, 1985; Glenney et al., 1987). The heavy chains of calpactin I, when not associated with light chains, are apparently cytoplasmic like calpactin II. The difference in localization of the monomeric and polymeric forms of calpactin I suggests that the light chain is required for membrane interaction, in agreement with the observation that the light chain increases affinity for phospholipids (Powell & Glenney, 1987). It also suggests that the two different pools of calpactin may have different functions or different mechanisms of action. Calpactin II and monomeric calpactin I turn over much faster than does the membrane-bound calpactin I (Zokas & Glenney, 1987).

Endonexin is partially membrane bound (Gerke & Weber, 1985b; Raeymakers et al., 1985) and may be associated with the endoplasmic reticulum (Geisow et al., 1984, 1986). The high molecular weight proteins, p70, are also partially associated with the plasma membrane of fibroblasts and lymphocytes (Geisow et al., 1984; Owens et al., 1984; Davies & Crumpton, 1985). In the electric organ, calelectrin is associated with intracellular membranes and is concentrated at the cytoplasmic face of synaptic vesicles (Fiedler & Walker, 1985). The dual, cytoplasmic and membranous, localization of these proteins is reminiscent of that of another Ca^{2+}- and phospholipid-binding protein, protein kinase C, and raises the possibility that Ca^{2+} controls both the distribution and the biological activity of these proteins. Studies of the relationship between intracellular Ca^{2+} concentration and localization of these proteins in intact cells would be helpful.

Physiological Role. Despite the progress made in the characterization of calpactins and related proteins, little is yet known about their functions or mechanisms of action. Their association with cytoskeleton proteins and localization suggest that they play a role in the organization of the network underlying the plasma membrane. However, the importance of association with the cytoskeleton may be questioned since antibodies to spectrin, which lead to spectrin aggregation, have no effect on the localization of calpactin (Mangeat & Burridge, 1984). Conversely, antibodies to calpactin light chains produce patching of both the light and heavy chains of calpactin without affecting fodrin distribution (Zokas & Glenney, 1987). These results, obtained after prolonged exposure to the antibodies, may not fully reflect the situation "in vivo" and therefore to do not definitively rule out a role for calpactin in the cytoskeleton, but they do raise questions about the significance of the association of these proteins with membrane and cytoskeleton proteins.

It has been proposed that Ca^{2+}-dependent membrane-binding proteins are involved in stimulus–secretion coupling on the basis of their abilities to promote membrane fusion (Creutz et al., 1978, 1981; Odenwald & Morris, 1983; Sudhof et al., 1982) and the bundling of actin filaments (Gerke & Weber, 1984; Glenney, 1986; Glenney et al., 1987). The high concentrations (millimolar) of Ca^{2+} required to observe these effects "in vitro" are not compatible with the much lower intracellular Ca^{2+} concentrations (in the micromolar range). However, more recent studies indicate that calpactins can promote fusion of chromaffin granules at micromolar Ca^{2+} (Drust & Creutz, 1988). In addition, the cell contains many other Ca^{2+}-binding proteins such as villin, fimbrin, gelsolin, and α-actinin (Weber & Glenney, 1985; Stossel et al., 1985), which may be required to constitute a system responding to physiological concentrations of Ca^{2+}.

At present the most attractive hypothesis is that calpactins are involved in the regulation of cell growth (and possibly cellular transformation) by acting as coupling agents between growth factor receptors and their cellular targets. Phosphorylation of calpactin II (p35) has been observed in vivo following exposure of the cells to EGF, and it was proposed to be involved in the internalization of the EGF receptor (Sawyer & Cohen, 1985). Attempts to correlate phosphorylation of calpactin II with its intracellular distribution have so far been fruitless. Although tyrosine phosphorylation of calpactin I decreases its affinity for liposomes in vitro (Powell & Glenney, 1987), phosphorylation of the protein in vivo does not seem to increase the amount of soluble calpactin (Zokas & Glenney, 1987). More quantitative experiments, preferably carried out in intact cells, are needed to reach more definitive conclusions. The affinity of the protein for the membranes could be too weak to detect differences in cellular distribution after disruption of the cells. The association of calpactin I with membranes may also be regulated by the extent of myristylation, which is decreased upon cellular transformation (Soric & Gordon, 1985).

A potentially important but not definitively demonstrated effect of phosphorylation is the loss of the ability of calpactin to inhibit phospholipase A_2 activity (Hirata et al., 1984). The physiological significance of the inhibition of phospholipase A_2 by lipocortins has been questioned by Davidson et al. (1987), who presented evidence that this effect is due to substrate sequestration rather than to a direct inhibition of the enzyme. Regardless of the mechanism underlying this inhibition, activation of the plasma membrane phospholipase A_2 (at present, only pancreatic and snake venom phospho-

```
Calpactin II   AMVSEFLKQAWFIENEEQEYVQTVKSSKGGPGSAVSPYPTFNP
                            ::  :   :  ::..
K-ras                      MTEYKLVVVGASGVGKSALTI------

Calpactin II   SSDVAALHKAIMVKGVDEATIIDILTKRNNAQRQQIKAAYLQETGKPLDETLKSALTGHLEEVVLALLKT
                                        : :  .. :   . .... .   :  .: .: :
K-ras          ---------------------------------QLIQNHFVDEYDPTIQDSYRKQVVIDGETCLLDILDT

Calpactin II   PAQFDADELRAAMKGLGTDEDTLIEILASRINKEIRDINRVYREELKRDLAKDITSDTSGDFRNALLSLA
                 : .  .: .  :    .. : ... .:     ::: :::   .        .  : :
K-ras          TGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHH----YREQLKRVKDSEDVPMVLVGNKCDLPS--

Calpactin II   KGDRSEDFGVNEDLADSDARALYEAGERRKGIDVNVFNTILITRSYPQLRRVFQKYTKYSKHDMNKVLDL
                  :. :     :: :   ..:  . .     : :..     :...         . : . .
K-ras          ---RIVDTKQAQELARSYGIPFIETSAKTRQRVEDAFYTLVREIRQYRLKKISKEEKTPGCVKIKKCVIM
```

FIGURE 3: Comparison of the sequences of human calpactin II and Kirsten murine sarcoma virus transforming protein (K-ras). The protein sequences of K-ras and calpactin II (residues 1–253) were compared with the ALIGN program of the NBRF by using the mutation data matrix and a break penalty of 10. The alignment score of 70 was five standard deviations above a randomized comparison of the sequences. The dashes indicate gaps inserted by the program. Identical residues (:) and conservative replacements (.) are as defined in legend to Figure 2. The consensus sequence of Geisow et al. (1986) is underlined.

lipases A_2 have been tested) by EGF in vivo following calpactin II phosphorylation could be of real significance to the physiology of growth factors.

Conclusions. Calpactin regulation of phospholipase A_2 catalyzed production of arachidonic acid would, if proven to be correct, bring support to the current proposal that arachidonic acid, like cAMP and Ca^{2+}, acts as a second messenger. There is evidence that arachidonic acid or one its metabolites regulates exocytosis (Barrowman et al., 1986; Burgoyne et al., 1987) and cell proliferation (Shier & Durkin, 1982; Vincentini et al., 1984). The proto-oncogene c-*ras*, which is required for initiation of DNA synthesis, is also needed for phospholipid-induced mitogenesis, suggesting that *ras* could link phospholipid metabolism with growth factor receptors (Mulcahy et al., 1985; Yu et al., 1988). Microinjection of H-*ras* protein activates phospholipase A_2 (Bar-Sadi & Feramisco, 1986). The mechanism of this effect is somewhat obscured by the fact that antibody to c-*ras* prevents the proliferative effects of phorbol esters, calcium ionophores, and prostaglandin F2a (Yu et al., 1987). In a recent search by the author, the only proteins in the NBRF data bank that were found to have significant sequence similarity with calpactin II were members of the *ras* family (Figure 3). The similarity includes a part of the GTP-binding site of the *ras* protein (residues 5–22 and 30–42); a stronger match is found elsewhere, in one of the repeat units of calpactin II. The similarity of calpactin II with β-transducin postulated earlier on the basis of antibody studies (Valentine-Braun et al., 1986) has been ruled out by Huang et al. (1986).

If calpactins are involved in the control of cell division, one would not expect them to be regulated by the transient Ca^{2+} signals induced by Ca^{2+}-mobilizing hormones since these hormones do not usually regulate cell proliferation. On the contrary, calpactins should be protected against these signals. The Ca^{2+} dependence may only reflect an additional modulation on top of a primary control exerted by phosphorylation as was suggested by Powell and Glenney (1987). Coupling between the two most commonly studied second messenger systems, cAMP and Ca^{2+}, is well established. Possibly similar coupling mechanisms exist that link the arachidonic acid pathway to cAMP and Ca^{2+}. Such a mechanism could explain the low Ca^{2+} concentration required to support growth by transformed cells.

ACKNOWLEDGMENTS

I thank Drs. Maxine Singer and Paul Wagner for helpful discussions and critical reading of the manuscript and Judy Glynn for expert secretarial assistance.

REFERENCES

Barrowman, M. M., Cockcroft, S. & Gomperts, B. D. (1986) *Nature (London) 319*, 504–507.

Bar-Sagi, D., & Feramisco, J. B. (1986) *Science (Washington, D.C.) 233*, 1061–1068.

Braslau, D. L., Ringo, D. L., & Rocha, V. (1984) *Exp. Cell Res. 155*, 213–221.

Burgoyne, R. D. (1987) *Nature (London) 328*, 112–113.

Cheng, Y. S.-E. & Chen, L. B. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2388–2392.

Cooper, J. A., & Hunter, T. (1985) *Annu. Rev. Biochem. 54*, 897–930.

Creutz, C. E., Pazoles, C. J., & Pollard, H. B. (1978) *J. Biol. Chem. 253*, 2858–2866.

Creutz, C. E., Dowling, L. G., Sando, J. J., Villar-Palasi, C., Whipple, J. H., & Zakz, W. J. (1983) *J. Biol. Chem. 258*, 14664–14674.

Crompton, M. R., Owens, R. J., Totty, N. F., Moss, S. E., Waterfield, M. D., & Crumpton, M. J. (1988) *EMBO J. 7*, 21–27.

Crouch, T. H., & Klee, C. B. (1980) *Biochemistry 19*, 3692–3698.

Davidson, F. F., Dennis, E. A., Powell, M., & Glenney, J. R., Jr. (1987) *J. Biol. Chem. 262*, 1698–1705.

Davies, A. A., & Crumpton, M. J. (1985) *Biochem. Biophys. Res. Commun. 128*, 571–577.

De, B. K., Misono, K. S., Lukas, T. J., Moczkowski, B., & Cohen, S. (1986) *J. Biol. Chem. 261*, 13784–13792.

Drust, D. S., & Creutz, C. E. (1988) *Nature (London) 331*, 88–91.

Erikson, E., Tomariewicz, H. G., & Erikson, R. L. (1984) *Mol. Cell. Biol. 4*, 77–85.

Fauvel, J., Vicendo, P., Ognes, V., Ragab-Thomas, J., Granier, C., Velgrain, I., Chambaz, E., Rochat, H., Chap, H., & Douste-Blazy, L. (1987) *FEBS Lett. 221*, 397–402.

Fava, R. A., & Cohen, S. (1984) *J. Biol. Chem. 259*, 2636–2645.

Fava, R. A., & Piltch, A. S. (1987) *J. Histochem. Cytochem. 35*, 1309–1315.

Fiedler, W., & Walker, J. H. (1985) *Eur. J. Cell Biol. 38*, 34–41.

Flower, R. J., Wood, J. N., & Parente, L. (1984) *Adv. Inflammation Res. 7*, 61–69.

Geisow, M. J., & Walker, J. H. (1986) *Trends Biochem. Sci. (Pers. Ed.) 11*, 420–423.

Geisow, M., Childs, J., Dash, B., Harris, A., Panayotou, G., Sudhof, T., & Walker, J. H. (1984) *EMBO J. 3*, 2969–2974.

Geisow, W. J., Fritsche, U., Hexham, J. M., Dash, B., & Johnson, T. (1986) *Nature (London) 320*, 636–638.

Gerke, V., & Weber, K. (1984) *EMBO J. 3*, 227–233.

Gerke, V., & Weber, K. (1985a) *J. Biol. Chem. 260*, 1688–1695.

Gerke, V., & Weber, K. (1985b) *EMBO J. 4*, 2917–2920.

Glenney, J. R., Jr. (1985) *FEBS Lett. 192*, 79–82.

Glenney, J. R., Jr. (1986a) *J. Biol. Chem. 261*, 7247–7252.

Glenney, J. R., Jr. (1986b) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4258–4262.

Glenney, J. R., Jr. (1987) *Biochem. Soc. Trans. 15*, 798–800.

Glenney, J. R., Jr., & Tack, B. F. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7884–7888.

Glenney, J. R., Jr., Boudreau, M., Gaylean, R., Hunter, T., & Tack, B. (1986) *J. Biol. Chem. 261*, 10485–10488.

Glenney, J. R., Jr., Tack, B., & Powell, M. A. (1987) *J. Cell Biol. 104*, 503–511.

Gould, K. L., Woodgett, J. R., Isacke, C. M., & Hunter, T. (1986) *Mol. Cell. Biol. 6*, 2738–2744.

Haigler, H. T., Schlaepfer, D. D., & Burgess, W. H. (1987) *J. Biol. Chem. 262*, 6921–6930.

Hayashi, H., Owada, M. K., Sonobe, S., Kakunaga, T., Kawakatsu, H., & Yano, J. (1987) *FEBS Lett. 223*, 267–272.

Hexham, J. M., Totty, N. F., Waterfield, M. D., & Crumpton, M. J. (1986) *Biochem. Biophys. Res. Commun. 134*, 248–254.

Hirata, F. (1984) *Adv. Inflammation Res. 7*, 71–78.

Hirata, F., Marsuda, K., Notsu, Y., Hattori, T., & del Carmone, R. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4717–4721.

Huang, C. Y., Chau, V., Chock, P. B., Wang, J. H., & Sharma, R. K. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 871–874.

Huang, K.-S., Wallner, B. P., Mattaliano, R. J., Tizard, R., Burne, C., Frey, A., Hession, C., McGray, P., Sinclair, E., Chow, E. P., Browning, J. L., Ramachandran, K. L., Tang, J., Smart, J. E., & Pepinsky, R. B. (1986) *Cell (Cambridge, Mass.) 46*, 191–199.

Isobe, T., & Okuyama, T. (1981) *Eur. J. Biochem. 116*, 79–86.

Johnsson, N., Vanderkerckhove, J., Van Damme, J., & Weber, K. (1986a) *FEBS Lett. 198*, 361–364.

Johnsson, N., Van, P. N., Soling, H. D., & Weber, K. (1986b) *EMBO J. 5*, 3455–3460.

Khanna, N. C., Tokuda, M., & Waisman, D. M. (1987) *Cell Calcium 8*, 217–228.

Kretsinger, R. H. (1980) *CRC Crit. Rev. Biochem. 8*, 119–174.

Kretsinger, R. H., & Creutz, C. E. (1986) *Nature (London) 320*, 573.

Kristensen, T., Saris, C. J. M., Hunter, T., Hicks, L. J., Noonan, D. J., Glenney, J. R., Jr., & Tack, B. F. (1986) *Biochemistry 25*, 4497–4503.

Kwok, Y. C., & Yip, C. C. (1987) *Biochem. J. 248*, 27–33.

Liu, Y., Brew, K., Carraway, K. L., & Carothers Carraway, C. A. (1987) *J. Cell. Biochem. 35*, 185–204.

Mangeat, P. H., & Burridge, K. (1984) *J. Cell Biol. 98*, 1363–1377.

Moore, P. B., & Dedman, J. R. (1982) *J. Biol. Chem. 257*, 963–967.

Mulcahy, L. S., Smith, M. R., & Stacey, D. W. (1985) *Nature (London) 313*, 241–243.

Odenwald, W. F., & Morris, S. J. (1983) *Biochem. Biophys. Res. Commun. 112*, 147–154.

Owens, J. R., & Crumpton, M. J. (1984) *Biochem. J. 219*, 309–316.

Owens, R. J., Gallagher, C. J., & Crumpton, M. J. (1984) *EMBO J. 3*, 945–952.

Pepinsky, R. B., & Sinclair, L. K. (1986) *Nature (London) 321*, 81–84.

Pepinsky, R. B., Sinclair, L. K., Browning, J. L., Mattaliano, R. J., Smart, J. E., Chow, E. P., Ribolini, A., Garwin, J., & Wallner, B. P. (1986) *J. Biol. Chem. 261*, 4239–4246.

Powell, M. A., & Glenney, J. R. (1987) *Biochem. J. 247*, 321–328.

Raeymakers, L., Wuytack, F., & Casteels, R. (1985) *Biochem. Biophys. Res. Commun. 132*, 526–532.

Rhoads, A. R., Lulla, M., Moore, P. B., & Jackson, C. E. (1985) *Biochem. J. 229*, 587–593.

Saris, C. J. M., Tack, B. F., Kristensen, T., Glenney, J. R., Jr., & Hunter, T. (1986) *Cell (Cambridge, Mass.) 46*, 201–212.

Sawyer, S. T., & Cohen, S. (1985) *J. Biol. Chem. 260*, 8233–8236.

Schlaepfer, D. D., & Haigler, H. T. (1987) *J. Biol. Chem. 262*, 6931–6937.

Shadle, P. J., & Weber, K. (1987) *Biochim. Biophys. Acta 897*, 502–506:

Shadle, P. J., Gerke, V., & Weber, K. (1985) *J. Biol. Chem. 260*, 16354–16360.

Shier, W. T., & Durkin, J. P. (1982) *J. Cell. Physiol. 112*, 171.

Smith, V. L., & Dedman, J. R. (1986) *J. Biol. Chem. 260*, 16354–16360.

Soric, J., & Gordon, J. A. (1985) *Science (Washington, D.C.) 230*, 563–566.

Stossel, T. P., Chaponniez, C., Ezzell, R. M., Hartwig, J. H., Janmey, P. A., Kwiatkowski, D. J., Lind, S. E., Smith, D. B., Southwick, F. S., Yin,H. L., & Zanez, K. S. (1985) *Annu. Rev. Cell Biol. 1*, 353–402.

Sudhof, T. C., Ebbecke, M., Walker, J. H., Fritsche, U., & Boustead, C. (1984) *Biochemistry 23*, 1103–1109.

Sudhof, T. C., Walker, J. H., & Fritshe, U. (1985) *J. Neurochem. 44*, 1302–1307.

Szebenyi, D. M. E., & Moffat, K. (1986) *J. Biol. Chem. 261*, 8761–8777.

Tokuda, M., Khanna, N. C., & Waisman, D. M. (1987a) *Biochem. J. 246*, 67–74.

Tokuda, M., Khanna, N. C., & Waisman, D. N. (1987b) *Methods Enzymol. 139*, 68–79.

Valentine-Braun, B. A., Northup, J. K., & Hollenberg, M. D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 236–240.

Vincentini, L. M., Miller, R. J., & Villereal, M. L. (1984) *J. Biol. Chem. 259*, 6912.

Walker, J. (1982) *J. Neurochem. 39*, 815–823.
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*, 77–81.
Weber, K., & Glenney, J. R., Jr. (1981) *Cold Spring Harbor Symp. Quant. Biol. 42*, 541–552.
Weber, K., Johnsson, N., Plessmann, U., Nguyen Van, P., Soling, H.-D., Ampe, C., & Vandekerckhove, J. (1987) *EMBO J. 6*, 1599–1604.
Yu, C.-L.; Tsai, M.-H., & Stacey, D. W. (1988) *Cell (Cambridge, Mass.) 52*, 63–71.
Zokas, L., & Glenney, J. R., Jr. (1987) *J. Cell Biol. 105*, 2111–2121.

Accelerated Publications

Cytochrome *b*-559 May Function To Protect Photosystem II from Photoinhibition†

Lynmarie K. Thompson and Gary W. Brudvig*
Department of Chemistry, Yale University, New Haven, Connecticut 06511
Received June 7, 1988; Revised Manuscript Received July 7, 1988

ABSTRACT: Although cytochrome *b*-559 is an integral component of the photosystem II complex (PSII), its function is unknown. Because cytochrome *b*-559 has been shown to be both photooxidized and photoreduced in PSII, one of several proposals is that it mediates cyclic electron transfer around PSII, possibly as a protective mechanism. We have used electron paramagnetic resonance spectroscopy to investigate the pathway of photooxidation of cytochrome *b*-559 in PSII and have shown that it proceeds via photooxidation of chlorophyll. We propose that this photooxidation of chlorophyll is the first step in the photoinhibition of PSII. The unique susceptibility of PSII to photoinhibition is probably due to the fact that it is the only reaction center in photosynthesis which generates an oxidant with a reduction potential high enough to oxidize chlorophyll. We propose that the function of cytochrome *b*-559 is to mediate cyclic electron transfer to rereduce photooxidized chlorophyll and protect PSII from photoinhibition. We also suggest that the chlorophyll(s) which are susceptible to photooxidation are analogous to the monomer chlorophylls found in the bacterial photosynthetic reaction center complex.

Naturally occurring intensities of visible light have been shown to cause inhibition of photosynthesis in vivo [reviewed by Powles (1984)]. When a photosynthetic organism is exposed to higher light levels than those to which it is adapted, or to some stress such as lack of water or carbon dioxide, it cannot catalyze electron transport fast enough to utilize all of the excitation energy it absorbs. The excess energy can cause reactions that damage the pigments (called photooxidation) or the photosystems (called photoinhibition). The most susceptible component of the photosynthetic apparatus to damage by light is photosystem II (PSII).[1] Photosynthetic organisms have several mechanisms for coping with excess excitation energy: changes in antenna size are used to limit or redistribute energy to prevent photoinhibition damage, and carotenoid pigments quench triplet chlorophyll, which sensitizes the formation of singlet oxygen, to prevent photooxidation damage (Powles, 1984). In this paper, we present an explanation for the unique susceptibility of PSII to photoinhibition and propose a mechanism that may be used in PSII for protection from this process.

The main electron transport chain in PSII is shown schematically in the box in Figure 1. Photoinhibition of PSII is thought to damage some component other than the oxygen-evolving complex, because artificial electron donors (which provide a source of electrons when the oxygen-evolving complex has been inactivated) do not restore electron-transfer activity (Powles, 1984). There is some controversy over the site that is damaged during photoinhibition. It has been suggested that the primary damage occurs at the Q_B site via reactions of oxygen radicals (Kyle et al., 1984; Kyle, 1987). This is based on evidence that photoinhibition decreases the binding of inhibitors to the Q_B site and decreases Q_B-dependent electron transfer. However, some laboratories have obtained conflicting results (Tytler et al., 1984; Callahan et al., 1986; Arntz & Trebst, 1986). One problem is that the mechanism of photoinhibition may vary with conditions such as anaerobicity (Krause et al., 1985), light intensity, and the intactness of the preparation. An alternate view is that photoinhibition damages some component involved in the primary charge separation; photoinhibition has been shown to inhibit photoreduction of Q_A (Cleland et al., 1986) and pheophytin (Demeter et al., 1987). Susceptibility to photoinhibition has also been correlated with conditions that prolong the lifetimes of $P680^+$ and

† This work was supported by the National Institutes of Health (Grant GM32715). G.W.B. is the recipient of a Camille and Henry Dreyfus Teacher/Scholar award (1985–1990) and an Alfred P. Sloan Foundation research fellowship (1986–1988). L.K.T. is the recipient of a dissertation fellowship from the American Association of University Women.

[1] Abbreviations: BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; Chl, chlorophyll; cyt b_{559}, cytochrome *b*-559; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; EPR, electron paramagnetic resonance; MES, 2-(*N*-morpholino)ethanesulfonic acid; Pheo, pheophytin; PQ, plastoquinone; PSII, photosystem II; P680, primary electron donor in PSII; P870, primary electron donor in reaction centers from *Rhodobacter sphaeroides*; Q_A and Q_B, primary and secondary quinone electron acceptors in PSII and the reaction center of purple non-sulfur bacteria; Z, secondary electron donor in PSII.

0006-2960/88/0427-6653$01.50/0 © 1988 American Chemical Society