Repeating sequence homologies in the p36 target protein of retroviral protein kinases and lipocortin, the p37 inhibitor of phospholipase A_2

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Received 12 May 1986

Although considerable information has emerged on the molecular properties of the p36 target protein its function as well as the possible implications of its tyrosine phosphorylation have remained elusive. Here we show that all sequence segments of p36 published so far can be aligned by homology along the complete sequence of lipocortin, which has been reported recently. This alignment extends beyond multiple Geisow motifs, thought to indicate a sequence principle implicated in Ca^{2+} and/or lipid binding. While the latter properties are already established for p36 one may expect them also for lipocortin, an inhibitor of phospholipase A_2 activity. Certain implications of these results are discussed.

Ca^{2+} (Intestine) Lipid Membrane protein Phospholipase Tyrosine phosphate

1. INTRODUCTION

Geisow, Walker, Suedhof and their colleagues have studied over the last few years a set of Ca^{2+}-dependent membrane-binding proteins possibly involved in exocytosis. For reasons only recently understood (see below) all these proteins reacted with a particular polyclonal antibody originally raised against a similar protein from the electric organ of *Torpedo*. Several experiments have led to the suggestion that these Ca^{2+}-dependent membrane-binding proteins could contain repeating sequences ([1–6]; earlier review [7]).

We found independently that microvillus membrane vesicles derived from the intestinal epithelium contain two Ca^{2+}-binding proteins in considerable amounts. These are lost from traditional brush border preparations with well-preserved ultrastructure due to the use of EGTA. One of these proteins, p36, was immediately recognized as the analogue of the fibroblastic p36 known to be a major cytoplasmic target of retrovirally coded tyrosine-specific protein kinases [8]. Because of the general interest in this target protein and its modification we and others continued to characterize p36 from intestinal epithelium where large-scale purification is easily possible [8,10]. p36 shows moderate Ca^{2+} binding, which causes the ability of the protein to bind to F-actin as well as to spectrin [8,10]. Certain lipids such as phosphatidylserine strongly enhance the Ca^{2+} binding and both ligands render p36 a better substrate for phosphorylation by viral protein kinases [11]. p36 can form a heterotetramer in the presence of a unique p11 protein [8,10]. p11 is by amino acid sequence highly homologous to the S100 protein ([12–14]; for complete sequence see [12]), a Ca^{2+}-binding protein of unknown function present primarily in glial cells. In spite of this relation direct binding of Ca^{2+} has not yet been found for p11 which, however, like S100 is a dimer [10,12]. In human epidermoid A431 cells, a p35 related [10] to p36 seems the prime target of the tyrosine kinase [15] associated with the EGF receptor and there is suggestive evidence that free p35

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00145793/86/$3.50

rather than the complex with p11 is the preferred target [10].

Having recently established two longer amino acid sequence stretches which characterized p36 as a unique protein and account for 23% of its length [16], we found no related protein in the sequence bank of the Biochemical Research Foundation. This conclusion is now changed by two papers published simultaneously with ours [1,17]. The complete c-DNA sequence of human lipocortin became available [17] and Geisow et al. determined the cross-reactivity of a particular antibody with several Ca^{2+}-dependent membrane-binding proteins [1]. They characterized short sequences which directly lead them to a consensus-type sequence. This seems to occur in several of these proteins more than once. As it is currently not known whether this motif reflects a novel Ca^{2+}-binding site or a lipid-binding site or both, we shall refer to this sequence principle as the Geisow motif. In a short review, published at the same time, Kretsinger and Creutz [18] drew attention to the presence of the motif in lipocortin. This protein, formerly called lipomodulin [19], is an inhibitor of phospholipase A_2 and thought to control the biosynthesis of the potent mediators of inflammation, the prostaglandins and leukotrienes (reviews [20,21]).

Here we show that the Geisow motif is present in p36 at least three times and also that p36 and lipocortin show extensive sequence homologies. Because of the ability to compare the longer sequences of p36 with lipocortin we are in a better situation to describe the Geisow motif than the earlier report covering only short segments [1].

2. RESULTS AND DISCUSSION

Although not noted by Wallner et al. [17] their human lipocortin sequence shows four consecutive segments with sequence homologies culminating in the motif region (fig.1). Three of these motifs which we call Lipo I, II and IV have been also noted by Kretsinger and Creutz [18]. They span residues 51–72, 123–144 and 282–303, respectively. The fourth site, Lipo III, situated around residues 207–228 may be functionally defective as a position always occupied by a large hydrophobic residue shows a hydrophilic asparagine.

Our two sequence segments [16] or porcine p36 align very well with human lipocortin (fig.1). Thus, the amino-terminally located segment, which we call p36 I, shows the Geisow motif and is in this region particularly closely related to Lipo I. Within the region 13 out of 24 residues are identical and there are several additional conservative replacements. This homology is higher than that seen when Lipo I and II are compared (11 identical residues). Our second sequence segment starting with the sole tryptophan of p36 can be aligned directly with the carboxy-terminal part of the motif. Although this alignment still requires consolidating sequences on the amino-terminal end, we note that our previous experiments implicate this tryptophan in the Ca^{2+}-induced difference spectra and fluorescence spectra [10,16]. Fig.1 shows that this segment, p36 III, which starts around residue 200 (see [16]) seems more closely related to Lipo III than to the other three motifs in lipocortin. Interestingly the short motif sequence of p36 reported by Geisow et al. [1] fits more closely to Lipo II and we have therefore tentatively labelled it p36 II. As the liver protein endonexin seems to have five repeating motifs [1] and lipocortin has four (see above) one might also expect a fourth motif for p36. Given the alignment with lipocortin shown in fig.1, such a putative site could be present relatively close to the carboxyl end of p36.

With more and longer sequences now available (fig.1) the motif sequences seem longer but display less absolute sequence requirements than thought earlier [1]. Invariant residues are a glycine and an arginine in positions 1 and 14 and two pairs of large hydrophobic residues present in positions 7, 8, 10 and 11. Positions 8 and 10 show a striking preference for isoleucine. Most likely position 4 involves in the majority a glutamic acid. Interestingly all four motifs of lipocortin and p36 III display a tyrosine 12 residue past the invariant arginine (fig.1). Future experiments have to determine whether it is this type of tyrosine which in p36 is influenced in its spectral properties by Ca^{2+} binding [10,16]. A similar tyrosine effect has been reported for a second protein of this group [22].

The particularly striking homology between p36 I and Lipo I seems to extend in a moderate way towards the amino-terminal side (fig.1) and this also seems true the other sites in lipocortin. Kretsinger and Creutz [18] raised the question as to

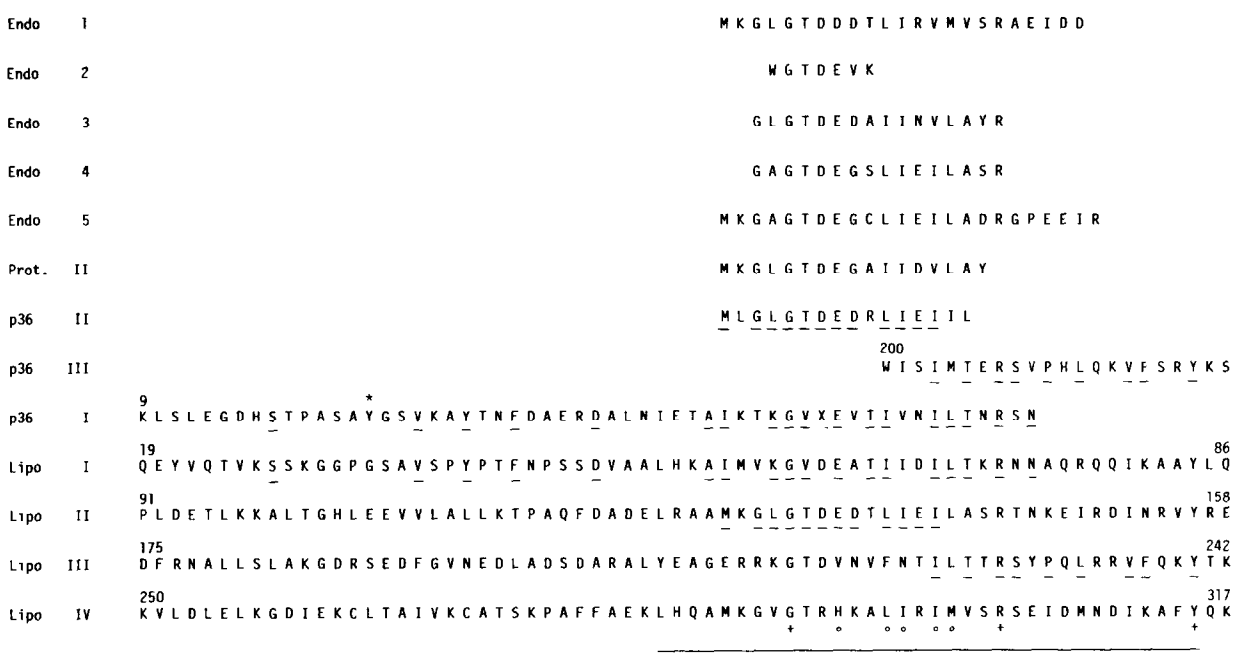

Fig.1. Sequence comparison between established longer fragments of porcine p36 and the sequence of human lipocortin. The four lipocortin sequences with corresponding residue numbers are taken from the complete sequence [17] and are labelled Lipo I–IV. The first five lines give the motif sequences found for bovine liver endonexin [1]. These are extended in lines 6 and 7 by short motif sequences reported for porcine intestinal epithelium protein II and porcine p36 from lymph nodes, respectively [1]. The two longer sequence segments labelled p36 I and III are from our study on p36 from intestinal porcine epithelium [16,25]. The approximate position of the Geisow motif is indicated by a line at the bottom. Note the invariant glycine and arginine in positions 1 and 14 indicated by + and two pairs of large hydrophobic residues at positions 7, 8, 10 and 11 indicated by a circle (see text). Also note that the longer sequences show a tyrosine 12 residues past the invariant arginine (circle). Residues underlined indicate identities between corresponding pairs of sequences: Lipo I|p36 I, Lipo II|p36 II and Lipo III|p36 III. Additional conservative replacements are not indicated to avoid overburdening the figure. Note, however, the corresponding homologies in the other sequences. Residue numbers for p36 are known only approximately [13,16,26]. For bovine p36 also the first 29 residues of p36 I are known [13]. They differ only by a proline (position 20) from the sequence given for the porcine protein [26]. The tyrosine phosphate is indicated by a star [13].

whether the position of phosphorylatable tyrosine residues could be related for both proteins. In p36 the tyrosine phosphate occurs [13] at position 23. Although phosphorylation of lipocortin [18,19] is thought to be functionally important, the position of the residue is not known. If it were located at either position 21 or 39 it would occur at a relatively similar distance from the first motif sequence in both proteins, although the surrounding sequences differ from each other and from those found in the auto-phosphorylation site of the protein kinases [9].

Whether the repeating motifs are indeed involved in Ca^{2+} and/or lipid binding will require studies on suitably small fragments or mutational studies on recombinant lipocortin. Although not established for lipocortin, certain other members of this family of proteins are already known to bind Ca^{2+} and for some of them multiple Ca^{2+} sites have been inferred from terbium fluorescence or Ca^{2+}-dependent spectroscopical studies [3,4,6,10,22–24]. Lipid binding is established at least for a few of these proteins [1,11,16] and it seems to involve always lipids present in the cytoplasmic leaflets of the plasma and endoplasmic reticulum membranes. Some proteins of the group have in addition protein ligands. Lipocortin is a phospholipase A_2 inhibitor [19–21]

and p36 binds to p11 and the cytoskeletal components F-actin and spectrin [8,10,12]. In p36 the major binding site for p11 resides in the loose domain preceding the first motif [16]. The presence of lipocortin in peritoneal exudates and culture medium of neutrophils seems to support the earlier notion that such proteins could be involved in exocytosis [2,6]. The presence of two related proteins in the microvilli of the intestinal epithelial cell [8] raises the question of whether they could also provide a submembraneous lining in a cellular extension otherwise stabilized by the core filament bundle of F-actin and its associated proteins (reviews [25,26]).

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