FEBS Letters 351 (1994) 171–175

FEBS 14467

Differential effects of fatty acid and phosholipid activators on the catalytic activities of a structurally novel protein kinase from rat liver

Nicholas A. Morrice[a], John Fecondo[b], Richard E.H. Wettenhall[a]

[a]*The Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Vic., 3052, Australia*
[b]*Department of Applied Chemistry, Swinburne University of Technology, 1 John Street, Hawthorn, Vic., 3122, Australia*

Received 25 June 1994; revised version received 20 July 1994

Abstract The lipid responsiveness of the structurally unique protein kinase, referred to as PAK-1, recently isolated from rat liver [(1994) J. Biol. Chem. 269, in press], is characterised by the high sensitivity (low micromolar) of its ribosomal S6(229–239) peptide kinase activity to both cardiolipin and the *cis*-unsaturated fatty acids and insensitivity to phosphatidylserine. Autophosphorylation of PAK-1 exhibited even greater sensitivity (submicromolar) to cardiolipin, but was relatively less affected by phosphatidylserine. Oleate, the most potent activator of PAK-1's peptide kinase activity was relatively ineffectual with autophosphorylation. These and other unusual characteristics, including high levels of basal catalytic activities, suggest a novel mechanism of regulation distinct from that of the protein kinase Cs

Key words: Lipid-activated protein kinase; Protease activation; Fatty acid; Cardiolipin; Autophosphorylation

1. Introduction

Recently, we reported the purification and characterisation of a structurally novel cardiolipin and protease-activated 116 kDa protein kinase from rat liver (referred to as PAK-1; [1]). Liver PAK-1 resembles the protein kinase C (PKC) isoenzymes with regard to its ability to efficiently phosphorylate ribosomal S6 peptide analogues [2], as well as its cardiolipin and protease activatability [1]. However, the enzyme displays different substrate preferences, is unresponsive to Ca^{2+} and phosphatidylserine/phorbol ester and is insensitive to the PKC-α(19–31) pseudosubstrate inhibitor peptide [1]. The complete amino acid sequence of liver PAK-1 [26] closely resembles that of the recently cloned human hippocampus and rat lung PKN sequence (99.5% identity) [3]. While the regulatory domain sequence is unrelated to that of any previously defined protein kinase, the amino acid sequence of its catalytic domain most closely resembles the corresponding sequences of the PKC isozymes (ca. 50% sequence identity). This similarity, together with the phospholipid sensitivity of PAK-1, suggests that the PKCs might serve as useful models for investigating the mechanism of regulation of liver PAK-1.

The PKCs are structurally and functionally a diverse family of enzymes which includes the calcium-dependent (α, βI, βII and γ) and independent (δ, ε, η and θ) and the atypical (ζ, λ and μ) isoenzymes [4–10]. All of the known PKCs are activated by phosphatidylserine, but not all are responsive to diacylglycerol or phorbol ester [5–8]. Other acidic phospholipids and various *cis*-unsaturated fatty acids can activate PKC isoenzymes with or without diacylglycerol (or phorbol esters) [5,9–11]. The calcium-independent and atypical isoenzymes, most resemble PAK-1 in that they exhibit less stringent dependency for phosphatidylserine and are more responsive to non-phosphatidylserine lipids than the calcium-dependent PKC isoenzymes [5,10].

Here we further define the responsiveness of liver PAK-1 to various acidic phospholipids and *cis*-unsaturated fatty acids both with respect to its peptide kinase and autophosphorylation activities.

2. Materials and methods

2.1. Materials

Phospholipids (PA, PG and Cl as sodium salts; PI as ammonium salt; PC, PS and PE as free lipids), fatty acids (free acid) and diacylglycerol were purchased from Sigma. [γ-^{32}P]ATP was from Bresatec Australia. Rat liver PAK-1 was purified to homogeneity as described previously [1] and assayed with the synthetic peptide, Ala^{229}-Lys-Arg-Arg-Arg-Leu-Ser-Ser-Leu-Arg-Ala-(NH2)^{239}, based on ribosomal protein S6 residues 229–239 [2]. The S6(229–239) peptide was synthesised on an Applied Biosystems 430A peptide synthesiser, using a simplified automated t-Boc solid phase peptide synthesis and reversed phase HPLC purification protocols based on the method of Schnölzer et al. 1992 [12], as modified by Mansell and Fecondo (in preparation). Brain PKC was prepared as described previously [13].

2.2. Experimental procedures

Lipids were prepared in chloroform, dried under a stream of nitrogen, and sonicated into 20 mM Tris-HCl, pH 7.5, at 20 μm for 4 × 15 s using an MSE sonicator. Dilutions were made in the same buffer with vigourous vortexing. Lipid-dependent PAK-1 activity was determined in a reaction mixture containing 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 40 mM KCl, 0.2 mM ATP (10–100 cpm/pmol), 30 μM S6(229–239), 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 2.5% (v:v) ethandiol, 0.0025% (v:v) Brij-35 (originating from storage buffer for purified PAK, [1]) and 0.1 mg/ml soyabean trypsin inhibitor, incubated with or without lipid additions for 10 min at 30°C as described previously [1].

2.3. Autophosphorylation of PAK-1

PAK-1 was incubated with 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 40 mM KCl_2, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 50 μM [γ-^{32}P]ATP (1,000–3,000 cpm/pmol), 0.0025% Brij-35 and 2.5% ethandiol, at 30°C for the indicated times in the presence and absence of lipids at the concentration specified in the figure legends. The fatty acid and phospholipid preparations were identified to those used in the S6(229–239) peptide kinase assays. The reaction was terminated by the addition of Laemelli SDS sample buffer [14] and the ^{32}P-labelled protein resolved by 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

*Corresponding author. Fax: (61) (3) 347 7730.

Abbreviations: PAK-1, protease activated protein kinase 1; PKC, protein kinase C; PS, phosphatidylserine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; CL, cardiolipin; PA, phosphatidic acid; TPA, 12-tetradecanoyl phorbol 13-acetate.

SSDI 0014-5793(94)00854-X

and subsequently detected by autoradiography using Kodak X-AR film and quantitated by densitometry (Molecular Dynamics Densitometer).

3. Results

3.1. Lipid activators of PAK-1 ribosomal S6 peptide kinase activity

Potential lipid regulators of purified liver PAK-1 were assessed using the ribosomal protein S6(229–239) peptide substrate, previously characterised as a low K_m (ca. 3.4 μM) substrate for the enzyme [1,2]. Initial screening with phospholipids and fatty acids at concentrations of 10 μg/ml and 20 μM, respectively, confirmed Ca^{2+}-independent activation of PAK-1 by acidic phospholipids other than phosphatidylserine, and showed the *cis*-unsaturated fatty acids also to be potent activators (Table 1). Several of the lipids, particularly oleic acid, arachidonic acid and cardiolipin, were even more effective than trypsin in the activation of the enzyme (Table 1). The saturated stearic and palmitic acids also stimulated activity, but to a lesser extent than the C18 to C20 unsaturated fatty acids (Table 1, Fig. 1). Fatty acid dosage dependency studies showed activation followed by inhibition of the PAK-1 peptide kinase activity (Fig. 1), a pattern resembling that observed previously with cardiolipin [1].

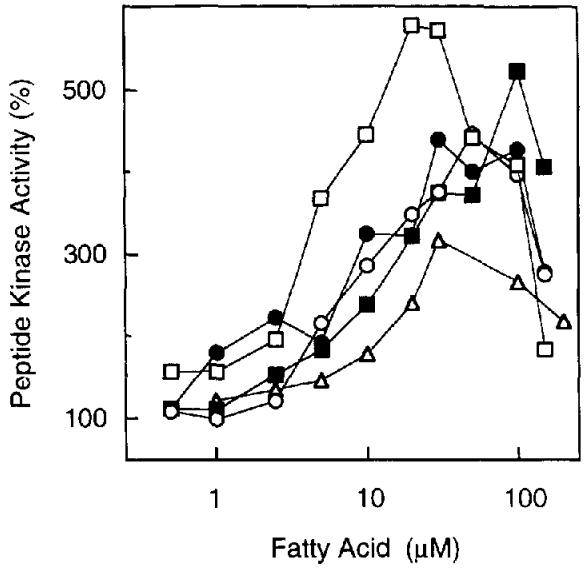

Fig. 1. Fatty acid dependency of PAK-1 peptide kinase activity: PAK-1 was assayed with 30 μM S6(229–239) as described in section 2 with sonicated dispersions of the following lipids; oleic acid (□), arachidonic acid (○), linoleic acid (●), linolenic acid (■) and stearic acid (△). Peptide kinase activity is expressed as a percentage of that obtained in the absence of lipids (100%).

Table 1
Effects of modifiers on PAK-1 activity

Modifier	Relative peptide kinase activity (%)	
	PAK-1	Brain PKC
None	100	–
10 μg/ml Cardiolipin	377	–
10 μg/ml Phosphatidylglycerol	344	–
10 μg/ml Phosphatidylcholine	100	–
10 μg/ml Phosphatidylethanolamine	100	–
10 μg/ml Phosphatidylinositol	282	–
10 μg/ml Phosphatidic acid	226	–
20 μM Stearic acid	240	–
20 μM Palmitic acid	175	–
20 μM Oleic acid	577	–
20 μM Arachidonic acid	348	–
Trypsin (standard PAK activation conditions)	330	–
1 mM EGTA	100	100
0.5 mM Ca^{2+}	86	219
100 μg/ml PS/1 mM EGTA	103	273
100 μg/ml PS/0.5 mM Ca^{2+}	90	2276
100 μg/ml PS/50 nM TPA/1 mM EGTA	99	1338
100 μg/ml PS/50 nM TPA/0.5 mM Ca^{2+}	90	2573
50 nM TPA/1 mM EGTA	86	151
50 nM TPA/0.5 mM Ca^{2+}	85	493

Purified PAK-1 was assayed for S6(229–239) peptide kinase activity without trypsin activation, with or without various phospholipids, fatty acids, TPA or $CaCl_2$ (Ca^{2+}). Peptide kinase activity (relative activity) was expressed as a percentage of that obtained for PAK-1 or brain PKC with no additions (6.1 and 3.3 pmol/min, respectively). The activity obtained with trypsin activation of this PAK-1 preparation (see reference for standard activation procedure) was 16.4 pmol/min and the activity obtained with phospholipid/TPA/Ca^{2+} activation of the brain PKC preparation was 84.9 pmol/min. These results are representative of a number of similar experiments with different preparations of PAK-1.

The stimulatory effects of the acidic phospholipids and *cis*-unsaturated fatty acids were only observed with sonicated preparations of lipids (Table 1) and were suppressed by the addition of the non-ionic detergent Triton X-100 at the low concentration (0.01%) sufficient to form the mixed micelles that support phosphatidylserine activation of the PKCs [15] (not illustrated). To eliminate the possibility that any effect of phosphatidylserine on PAK-1 was specifically interferred with by the detergent Brij-35 (0.0025%) present in the standard PAK assay reaction mixture (see section 2), the screening lipids was carried out in the absence of detergent (cf. [1]). Under these conditions, the phosphatidylserine preparation had no effect on PAK-1 peptide kinase activity, but was fully effective in the activation of brain PKC (20–30-fold stimulation) (Table 1). The relatively greater maximal stimulation of brain PKC by phospholipid (20–30-fold), compared with the 3–4-fold lipid stimulation of PAK-1 activity under the same conditions, is a characteristic difference between the two enzymes (Table 1). This primarily reflected the relatively low level of basal activity of PKC preparations, with the specific activities of the fully lipid-activated PAK-1 and PKCs being of the same order of magnitude (Table 2) [1]. Unlike the PKCs, the activation of PAK-1 by either the phospholipids or fatty acids did not display synergy or additive effects with either diacylglycerol or TPA (not illustrated) [1], and the addition of either of these activators had no effect in the presence of phosphatidylserine (Table 1).

The V_{max} values for S6 peptide phosphorylation showed that the C18 and C20 *cis*-unsaturated fatty acids were the most potent overall activators of PAK-1 (Table 2). The lower V_{max} values for the saturated stearic and palmitic acids, and the various acidic phospholipid activators, i.e. <65% of the values

for oleic acid (Table 2), suggested that the inhibitory effects of higher concentrations of lipids (e.g. Fig. 1) [1] became dominant before full activation of the enzyme was achieved. While overall oleic acid was the most effective of all of the activators tested, the EC_{50}s for cardiolipin and phosphatidylinositol (1.7 and 3.1 μM, respectively) were lower than for oleic acid (4.2 μM) (Table 2). This indicated that these phospholipids interated with PAK-1 at least as efficiently as oleic acid. Their inability to elicit full activation may have reflected the formation of inhibitory higher order lipid structures below the lipid concentration required for full activation.

A comparison of the EC_{50} values for the series of C18 fatty acids established that a critical structural feature determining the sensitivity of PAK-1 was the C9–10 double bond, the introduction of which (i.e. oleic versus stearic acids) caused a >3-fold reduction in the EC_{50} and increased the maximal activation nearly 2-fold (Table 2). Additional double bonds had a negative influence with the EC_{50}s increasing from 4.2 μM for oleic, to 8.0 μM for linoleic, to 19.7 μM for linolenic (Table 2). The relatively high EC_{50}s of 72.1 μM for palmitic acid compared with 14.3 μM for stearic acid, (Table 2), indicated that carbon chain length is also an important determinant of the efficacy of fatty acids as PAK-1 activators.

3.2. *Differential effects of lipids on PAK-1 autophosphorylation*

To further assess the lipid responsiveness of PAK-1, the effects of the various lipid activators of peptide kinase activity on the autophosphorylation activity of the enzyme were investigated, thus eliminating potential complications due to lipid-peptide substrate interactions as can occur with PKCs [16]. Incubation of purified PAK-1 with [γ-^{32}P]ATP resulted in the rapid autophosphorylation of the 116 kDa PAK-1 polypeptide (Fig. 2). In the absence of any activators, autophosphorylation approached a maximum stoichiometry of about 1 mol phosphate per mol enzyme within 30 min of incubation (Fig. 3; time course not illustrated). Both the rate (not illustrated) and stoichiometry (Figs. 2 and 3) of PAK-1 autophosphorylation were stimulated by cardiolipin. While the magnitude of the cardiolipin effect varied with different preparations of enzyme and lipid (stimulations ranged from 2.9- to 3.7-fold in the experiments described in Figs. 2 and 3), in each of the experiments cardiolipin stimulated autophosphorylation to a greater extent than any of the other lipid activators tested (Fig. 2). Dosage dependence studies showed that maximum autophosphorylation was achieved at a cardiolipin concentration of about 20 μM, with a sharp decrease in activity at higher concentrations, thus resembling the previously described cardiolipin curve for peptide phosphorylation [1] (Fig. 3). However, autophosphorylation was more sensitive to cardiolipin with appreciable effects (1.7–2.2-fold stimulation) evident at concentrations as low as 0.1 μM, whereas comparable effects on peptide phosphorylation required 10-fold higher concentrations (>1 μM) (Fig. 3).

Table 2
Lipid dependence of PAK-1

Lipid	EC_{50}	V_{max} (μmol/min/mg)
Cardiolipin	1.7 μM	3.67
Phosphatidylinositol	3.1 μM	3.03
Phosphatidylglycerol	7.8 μM	3.40
Phosphatidic acid	15.9 μM	3.28
Oleic acid	4.2 μM	5.62
Arachidonic acid	10.1 μM	4.34
Linoleic acid	8.0 μM	4.28
Linolenic acid	19.7 μM	5.09
Palmitic acid	72.1 μM	3.40
Stearic acid	14.3 μM	3.09

PAK-1 was assayed with the following lipids using 30 μM S6(229–239) as substrate. The concentration yielding half maximal activity of PAK-1 is expressed as an EC_{50} value. Phospholipid concentrations were calculated assuming that the M_r values for the following analogues approximated to the average M_r values of the corresponding natural phospholipid preparations. Cardiolipin (tetra linoleyl; di-sodium salt, M_r = 1491.9), phosphatidylinositol (1-stearic, 2-arachidonyl; ammonium salt, M_r = 906.2), phosphatidylglycerol (1,2-dioleoyl; sodium salt, M_r = 797) and phosphatidic acid (1,2-dimyristyl; sodium salt, M_r = 614.8).

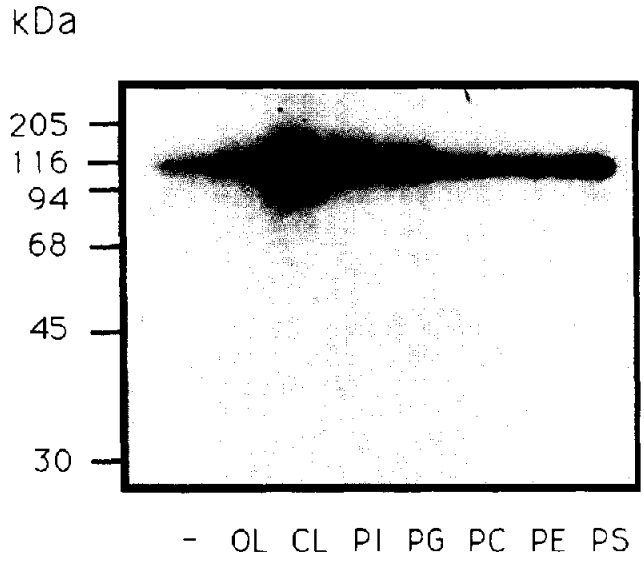

Fig. 2. Lipid dependency of PAK-1 autophosphorylation: PAK-1 (0.1 μg) was incubated in the presence of 50 μM ATP for 20 min with either 10 μM fatty acid or 20 μg/ml phospholipid; no addition (−), oleic acid (OL), cardiolipin (CL), phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS). ^{32}P-Labelled PAK-1 was resolved by 7.5% SDS-PAGE and detected by autoradiography. The relative percentage of PAK-1 autophosphorylation determined from densitometry were; control, 100%; OL 170%, CL 374%, PI 330%, PG 310%, PC 190%, PE 176% and PS 200%.

Phosphatidylinositol and phosphatidylglycerol also stimulated PAK-1 autophosphorylation (3.3- and 3.1-fold, respectively), to nearly the same extent as cardiolipin (3.7-fold; Fig. 2). Autophosphorylation was appreciably less affected by phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine (2.0-, 1.9- and 1.7-fold stimulation, respectively) (Fig. 2). Oleic acid, the most effective activator of S6 peptide phosphorylation (Fig. 1), was also relatively ineffectual as a stimulator of autophosphorylation (1.7-fold) under assay conditions similar to those where the full effect of the fatty acid on the enzyme's S6 peptide kinase activity (5.8-fold) was evident (Fig. 2, cf. Fig. 1).

4. Discussion

Liver PAK-1's unique structure, moderate abundance, restricted sensitivity to phospholipid regulators and substrate

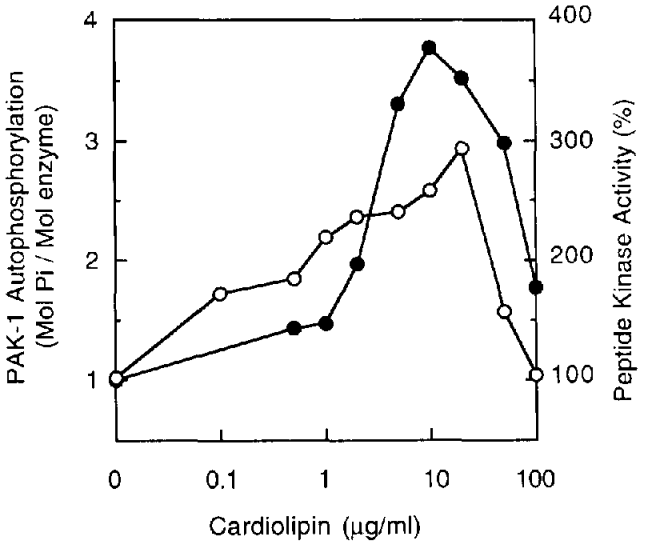

Fig. 3. Cardiolipin dependency of PAK-1 autophosphorylation activity: PAK-1 autophosphorylation (○) or S6(229–239) peptide kinase (●) activities were assayed in the presence of 0–100 μg/ml cardiolipin. Representative peptide kinase data taken from experiment reported previously [1] are shown for comparison. Autophosphorylation was assayed in the presence of 50 μM ATP for 20 min at 30°C and S6 peptide kinase activity was assayed as in section 2. The stoichiometry of autophosphorylation was quantitated by excising gel pieces containing PAK-1 and determining the ^{32}P-radioactivity by liquid scintillation counting.

specificity suggests an important role in hepatic regulation [1]. The regulatory properties of the enzyme are characterised by unusually high sensitivities to cardiolipin and C18 to C20 *cis*-unsaturated fatty acids, as well as differential lipid specifities towards activation of its S6 peptide kinase and autophosphorylation activities. The lipid specificities, although overlapping, are distinct from the specificities observed for any of the PKCs particularly in relation to phosphatidylserine responsiveness [4,6–8]. These properties and the unique structural features of its regulatory domain [26] suggest that liver PAK-1, together with the structurally similar protein kinases encoded by the recently described PKN cDNAs [3], are members of a new class of protein kinase within a super family of phospholipid-dependent protein kinases [1]. Of particular interest is the absence of the functional motifs characteristic of the PKC regulatory domains [3,26], including the zinc finger motifs implicated in diacylglycerol binding [4,6–8] and the PKC pseudosubstrate motifs directly involved in the intramolecular inhibition of PKCs in the absence of phospholipid activators [17]. These structural differences can explain PAK-1s unresponsiveness to diacylglycerol and phorbol ester, as well as its basal S6 peptide kinase [1] and autophosphorylation (this paper) activities, which are characteristically high compared with the relatively very low basal activities of the PKCs [8–11]. A lack of a fully functional binding site for phosphatidylserine, analogous to the still undefined structural motif which determines the high specificity of the PKCs for the phosphatidylserine head group [4,6], could explain the insensitivity of the enzyme's S6 peptide kinase activity to this lipid. The interaction with phosphatidylserine, resulting in limited activation of PAK-1 autophosphorylation by phosphatidylserine, only equivalent to that elicited by the basic phospholipids, and, insufficient to affect exogenous substrate phosphorylation, suggests non-specific interaction through binding to a functional site, possibly related to one of the sites targetted by cardiolipin or fatty acids [19]. In the case of the PKCs, there is evidence for more than one type of oleate binding site, at least, one of which is suppressed by phosphatidylserine binding [19].

The lipid responsiveness of PAK-1, in vitro, suggests that the regulation of the enzyme, in vivo, involves some form of lipophilic second messenger. While cardiolipin is unlikely to be a physiological regulator, given its exclusive location within the inner mitochondrial membrane [18], it could mimmick the action of a more physiologically relevant phospholipid activator(s). Candidates include phosphatidic acid which can also activate PAK-1, *in vitro*, although not as effectively as cardiolipin (Table 2, [1]), and which has been implicated as a second messenger in mammalian signal transduction [18–20]. Other potential physiological regulators include the *cis*-unsaturated fatty acids shown here to activate PAK-1 at concentrations (low micromolar) within the physiological range in liver. The high sensitivity to oleic acid distinguishes PAK-1 from the classic PKCs which require relatively higher concentrations for maximal activation, are usually more responsive to arachidonic acid and usually exhibit synergy between fatty acids and diacylglycerol and phosphatidylserine [18,21,22]. The relatively high specificity for oleate compared with other C18 fatty acids, further suggests oleate as an intracellular regulator of PAK-1. The enzyme most resembling PAK-1 with respect to oleate (as well as phosphatidic acid) sensitivity is the novel lipid-dependent protein kinase recently isolated from human platelets, for which maximun activation is achieved at ca. 10 μM oleate [19].

The differential effects of the most potent activators of exogenous substrate kinase activity, cardiolipin and oleic acid, on autophosphorylation suggest different modes of regulation of PAK-1. The different modes may relate to the two compartment model for the PKCs proposed by Hannun and co-workers [23], whereby phospholipids and diacylglycerols activate membrane bound PKCs and fatty acids selectively activate cytoplasmic forms of PKCs. Differential autophosphorylation events in response to the different activators may help to maintain the enzyme in a particular compartment, as well as influence substrate specificities and/or affinities as appears likely in the case of the PKCs. Accordingly, the extensive autophosphorylation associated with the phospholipid-type activation of PAK-1 and the PKCs [6,24] may facilitate the partitioning of the enzymes to intracellular membranes [25], while the relatively low autophosphorylation associated with oleate activation of PAK-1 may maintain an active cytoplasmic form. Maximal activation by oleate in the absence of an effect on autophosphorylation has previously been observed with platelet PKC-α [21], suggesting that this phenomenon could be a general feature of fatty acid activation of protein kinases. In this context, it will be of interest to determine whether the differential targetting of individual PKCs to different intracellular sites following activation [23] and the selective partitioning of the PAK-1-related recombinant PKN [3] to the nuclear fraction of COS 7 cells, is influenced by autophosphorylation events.

Acknowledgments: This work was funded by a grant from the National Health and Medical Research Council of Australia. We wish to thank Professor Bill Sawyer and Dr. Bruce Grant and Professor Peter Quinn

for their gifts of lipids and helpful advice and Miss Helen Varnavas for the preparation of this manuscript.

References

[1] Morrice, N.A., Gabreilli, B.J., Kemp, B.E. and Wettenhall, R.E.H. (1994) J. Biol. Chem. 269 (in press)
[2] Wettenhall, R.E.H., Gabrielli, B., Morrice, N.A., Bozinova, L., Kemp, B.E. and Stapleton, D. (1992) Peptide Res. 4, 158–170
[3] Mukai, H. and Ono, Y. (1994) Biochem. Biophys. Res. Commun. 199, 897–904
[4] Azzi, A., Boscoboinik, D. and Hensey, C. (1992) Eur. J. Biochem. 208, 547–557
[5] Nakanishi, H. and Exton, J.H. (1992) J. Biol. Chem. 267, 16347–16354
[6] Newton. A.C. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 1–35
[7] Nishizuka, Y. (1988) Nature 334, 661–665
[8] Osada, S.I., Mizuno, K., Saido, T.C., Suzuki, K., Kuroki, T. and Ohno, S. (1992) Mol. Cell. Biol. 12, 3930–3938
[9] Schaap, D. and Parker, P.J. (1990) J. Biol. Chem. 265, 7301 7307
[10] Saido, T.C., Mizuno, K., Konno, Y., Osada, S.-I, Ohno, S. and Suzuki, K. (1992) Biochemistry 31, 482–490
[11] Sekiguchi, K., Tsukuda, M., Ase, K., Kikkawa, U. and Nishizuka, Y. (1988) J. Biochem. 103, 759 765
[12] Schnölzer, M., Alewood, P.F., Jones, A., Alewood, D. and Kent, S.B.H. (1992) Int. J. Pept. Prot. Res. 40, 180–193.
[13] House, C., Wettenhall, R.E.H. and Kemp, B.E. (1987) J. Biol. Chem. 262, 772–777
[14] Laemelli, U.K. (1970) Nature 227, 680–685
[15] Hannun, Y.A., Loomis, C.R and Bell, R.M. (1985) J. Biol. Chem. 260, 10039–10043
[16] Bazzi, M.D. and Nelsestuen, G.L. (1987) Biochemistry 26, 5002 5008
[17] House, C. and Kemp, B.E. (1987) Science 238, 1726–1728
[18] Bell, R.M. and Burns, D.J. (1991) J. Biol. Chem. 266, 661–4664.
[19] Khan, W.A., Blobe, G.C., Richards, A.L. and Hannun, Y. (1994) J. Biol. Chem. 269, 9729–9735
[20] Martin, A., Gomez-Muñoz, A., Waggoner, D.W., Stone, J.C. and Brindley, D.N (1993) J. Biol. Chem. 268, 3924–23932.
[21] Touny, S.E., Khan, W. and Hannun, Y. (1990) J. Biol. Chem. 265, 16437–16443
[22] Chen, S.G. and Murakami, K. (1992) Biochem. J. 282, 33–39.
[23] Khan, W.A., Blobe, G.C. and Hannun, Y. (1992) J. Biol. Chem. 267, 3605–3612
[24] Newton, A.C. and Koshland Jr., D.E. (1987) J. Biol. Chem. 262, 10185–10188
[25] Huang, K.P., Chan, K.F., Singh, T.J., Nakabayashi, H. and Huang, F.L. (1986) J. Biol. Chem. 261, 2134–12140.
[26] Peng, B., Morrice, N.A., Rasmussen, R. and Wettenhall, R.E.H., in preparation.