A homologue of human protein kinase C (PKC)-related kinase-2, PRK2, which had previously escaped identification in normal mammalian tissues, was isolated from rat liver as the protease-activated kinase (PAK) originally named PAK-2. The 130-kDa cytosolic enzyme was purified to homogeneity and shown by tryptic peptide and reverse transcriptase-polymerase chain reaction (RT-PCR)-amplified rat cDNA sequence analyses to be structurally related to the 116-kDa rat hepatic PK-1/protein kinase N (PRK) and, even more closely (95% sequence identity) to the 130-kDa human PKC-related kinase, PRK2. Rat myeloma RNA was used as the RT-PCR template because of its relative abundance in PAK-2/PRK2 mRNA compared with liver and other rat tissues. The catalytic properties of PAK-2/PRK2 in many respects resembled those of hepatic PK-1/PRKN, but were distinguished by more favorable kinetics with several peptide substrates, and greater sensitivity to PKC pseudosubstrate and polybasic amino acid inhibitors. PAK-2/PRK2 was also activated by lipids, particularly cardiolipin and to a lesser extent by other acidic phospholipids and unsaturated fatty acids. Cardiolipin activation was most evident with autophosphorylation and histone H2B phosphorylation, but only marginally evident with the favored ribosomal S6-(229–239) peptide substrate for the protease-activated kinase activity. It was concluded that PAK-2 is the rat homologue of human PRK2, with biochemical properties distinct from although overlapping those of the PAK-1/PRKN/PRK1 isoform.

**Isolation and Characterization of a Structural Homologue of Human PRK2 from Rat Liver**

**DISTINGUISHING SUBSTRATE AND LIPID ACTIVATOR SPECIFICITIES**

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Lipid-regulated protein kinases, best characterized by the protein kinase Cs (PKCs)\(^1\) (1–3), also include the recently discovered family variously named the liver PAK\(^2\) (4, 5), PKN (6–8), and the PKC-related kinases (PRKs) (9–11). While several PKN/PRK isoforms have been detected by cDNA/PCR analyses (9), direct mRNA analyses have detected only the ubiquitously expressed PKN/PRK1 in human and rat tissues (6, 9), PRK-3 in human myeloma and a few rat tissues (9), and PRK2 only in human myeloma cell lines (9). PKN/PRK1-type isoforms have been isolated and structurally characterized, originally as the naturally occurring liver protease-activated kinase, PAK-1 (4, 5), and, subsequently as the cDNA-derived recombinant proteins (6, 9, 12). Isoforms of this type share several biochemical characteristics with the PKCs, particularly their general substrate preferences and activatability by proteases, phospholipids and unsaturated fatty acids (4, 5, 7, 8, 10, 11, 13), but are distinguished from the PKCs by their phospholipid specificity (5, 13), inhibitor sensitivity (5), substrate kinetics (5), and activatability by the RhoA GTP-binding protein (14, 15). Human PRK1 and PRK2 share structurally very similar catalytic domains (87% identity), but relatively less similar N-terminal regulatory regions (48% identity; see Palmer and Parker (9)), suggestive of different regulatory domain functions.

The failure to detect PRK2 mRNA in normal human and rat tissues suggests either that expression is restricted to abnormal cell membranes of the myeloma type (9) or that low abundance or an intrinsic structural feature of the mRNA prevents its ready detection. Direct screening of rat liver extracts (cytosol) for PRK-like enzyme activities has identified a second protease-activated kinase (PAK-2) with biochemically similar, although nonidentical, properties to those of liver PAK-1/PRKN (5, 16). This investigation concerns the purification and characterization of this enzyme, including an assessment of its relationship with the PRKs.

**EXPERIMENTAL PROCEDURES**

**Materials**—\(^{[\gamma-^32P]}\)ATP and synthetic oligonucleotides were purchased from Bresatec Ltd. (Australia); DE-52 cellulose and P81 paper from Whatman (UK); all other chromatographic supports from Pharmacia LKB (Sweden); ethanediol (Analar) from Merck BDH (UK); protease inhibitors, protein substrates, phospholipids, fatty acids, and Brij-35 from Sigma; Trizol total RNA isolation reagent from Life Technologies, Inc.; Taq DNA polymerase from Boehringer Mannheim (Germany); murine leukemia virus reverse transcriptase and ABI PRISM dye terminator cycle DNA sequencing kit from Perkin-Elmer. Synthetic peptides were synthesized (as C-terminal amides) on an Applied Biosystems 430A peptide synthesizer as described previously (13, 17). The following reagents were prepared as described previously: threonine-Sepharose 4B (18), protamine CH-Sepharose 4B (9), and bovine serum Leu-Arg-Arg-Leu; CL, cardiolipin; PCR, polymerase chain reaction; RT, reverse transcriptase; PMSF, phenylmethylsulfonyl fluoride.

\(^{2}\) The often used abbreviation PAK to describe protease-activated kinase (e.g. see Refs. 4, 5, and 19) should not be confused with the recent use of the same abbreviation to describe p21 protein-activated kinases (e.g. see Manser et al. (29)).
albun (essentially fatty acid-free) treated with PMSF (19).

Purification of PAK-2 from Rat Liver—All procedures were carried out at 4 °C. The liver postmicrosomal (150,000 × g) supernatant fraction of 4–6 months-old Buffalo rats, fed ad libitum, was prepared as described previously (5) and chromatographed by batch elution on DE-52 cellulose (50 ml/100 g of tissue) equilibrated in buffer A (200 mM Tris-HCl, pH 8.5, 1 mM EDTA, 2 mM EGTA, 10 mM 2-mercaptoethanol, 0.25 mM PMSF, and 1 mM benzamidine). The PAK activity was eluted with 2 column volumes of buffer A containing 0.15 M NaCl, the eluate diluted with 2 volumes of buffer B (150 mM potassium phosphate, pH 6.5, 1 mM EDTA, 2 mM EGTA, 10 mM 2-mercaptoethanol, 0.25 mM PMSF, and 1 mM benzamidine) and adjusted to pH 6.5 by addition of 0.1 M of 150 mM potassium phosphate, pH 6.5, and applied to a CM-Sephadex column (50 ml/100 g of tissue) equilibrated with buffer B. The column was washed with 4 volumes of buffer B and batch eluted with 2 volumes of buffer B containing 0.32 M KCl, followed by 2 volumes of buffer B containing 0.3 M KCl. The PAK-2 activity recovered in the 0.3 M KCl eluate was further purified by threonine-Sepharose 4B and protamine-CH-Sepharose as described previously for liver PAK-1 (5), except that leupeptin (2 µg/ml) was included in all buffers. The post-

Protein Kinase Assays—Aliquots (10 µl) of column fractions were subjected to mild trypsinolysis and the trypsin-activated kinase activities determined using a reaction mixture (60 µl) incubated for 15 min at 30 °C, as described previously (5, 19). Peptide substrate(s) were used at 30 µM, protein substrates at 0.2 mg/ml, and ATP at 200 µM (10-fold higher than its K_m of 19 µM); the MgATP ion concentration of 5 mM was within the optimal range for PAK-2 phosphotransferase activity (2 to 6 mM). Peptide phosphorylation was determined by the Whatman P81 paper-binding assay, as described previously (5). The effects of sonicated phospholipid and fatty acid dispersions on PAK-2 activity were investigated using the same reaction mixture except that the trypsin treatment was omitted (5, 13). One unit of peptide kinase activity is defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of phosphate into S6-(229–239) peptide/min. Reaction conditions and data analyses for the determination of K_m and V_max values, were as described previously for liver PAK-1/PKN (5); values reported represent the means of three independent experiments.

RESULTS AND DISCUSSION

Purification of 130-kDa PAK-2 from Rat Liver—The effectiveness of the purification strategy involving sequential column chromatography steps based on DE-52 (4, 5), CM-Sephadex (4, 5), threonine-Sepharose, protamine-Sepharose, Mono S and Mono Q, is illustrated by the SDS-PAGE analysis of PAK-2 peak fractions at each step (Fig. 1). The rapid batchwise elution in the DE-52 and CM-Sephadex steps minimized losses of PAK-2 activity due to its high susceptibility to proteolysis by endogenous proteases (5). The PAK-2 activity eluted from Mono S was clearly resolved from the earlier eluting residual PKA-1 (not illustrated). In the final Mono Q step, the single peak of PAK-2 activity (Fig. 2A) copurified with a discrete 130-kDa polypeptide (Fig. 2C). Confirmation that the 130-kDa polypeptide corresponded to the protein kinase was obtained by analysis of the autophosphorylation activity of the Mono Q column fractions by SDS-PAGE and subsequent autoradiography (Fig. 2B).

Primary Structural Analysis of PAK-2—To establish the structural identity of PAK-2, tryptic peptides generated from pyridylethylated PAK-2 were separated by reversed phase-HPLC (not illustrated) and subjected to amino acid sequence analyses (Fig. 3). Screening of the SwissProt and GenBank data bases established that, of the 17 tryptic peptide se-

The following protein and nucleic acid sequence data bases were used as sources for protein kinase sequences: Swiss Prot, European Molecular Biology Laboratory, Postfach 102299, D-6900 Heidelberg, Germany; GenBank, National Center for Biotechnology Information, National Library of Medicine, Building 38A, Room 8N-805, 8600 Rockville Pike, Bethesda MD 20894.

Fig. 1. SDS-PAGE analysis of PAK-2 fractions during purification. Fractions of each chromatography step were subjected to 7.5% SDS-PAGE analysis. Proteins were visualized by silver staining. Lane 1 (molecular markers as indicated in the right side of the gel); lane 2 (DEAE-cellulose) 1.9 µg; lane 3 (CM-Sephadex) 1 µg; lane 4 (threonine-Sepharose) 0.6 µg; lane 5 (prolamine-Sepharose) 0.32 µg; lane 6 (Mono S) 0.23 µg; lane 8 (Mono Q) 0.075 µg.

FIG. 2. Mono Q chromatographic analysis of PAK-2 activity. PAK-2 Mono Q column fractions were assayed for S6-(229–239) peptide kinase (A) and autophosphorylation (autoradiography of SDS-PAGE gel) (B) activities and analyzed by SDS-PAGE with Coomassie Blue staining (C), as described under "Experimental Procedures."

RT-PCR Amplification and DNA Sequencing—Total RNA fractions from several rat tissues and the rat myeloma Y3 cell line (20) were prepared using the Trizol reagent-based extraction procedure (as described by the manufacturer) and screened for RT-PCR amplification (21) based on sequence-specific PRK2/PAK-2 probes. The first strand cDNA was synthesized in a reaction based on 250 ng of total RNA, oligo(dT) primer, and murine leukemia virus reverse transcriptase. Subsequent PCR amplification of overlapping cDNA template sequences with Thermus aquaticus (Tag) DNA polymerase was based on degenerate oligonucleotide primers corresponding to either PAK-2 tryptic peptide-derived or PKN/PRK2-conserved sequences. The gel-purified PCR-amplified DNA products were sequenced on an Applied Biosystems 373 DNA sequencer using ABI PRISM dye terminator chemistry as instructed by the manufacturer.

Other Procedures—PAK-2 autophosphorylation was assayed as described for liver PAK-1 (13), except that the ATP concentration was 200 µM and 15 µg PMSF-treated bovine serum albumin was included in the 50-µl reaction mixture as a stabilizing agent. Purified PAK-2 was alkylated with 4-vinylpyridine and digested with alkylated trypsin, and the tryptic peptides were separated by reversed phase-HPLC and sequenced, as described previously for liver PAK-1 (5). Protein concentrations were measured by the Bradford assay (22). SDS-PAGE was performed as described by Laemmli (23), with Coomassie Blue (23) or silver (Bio-Rad) (24) staining.
sequences identified (Fig. 3), four (T14–T17) displayed appreciable similarity with the catalytic domain consensus sequences of rat PAK-1/PKN, its C-terminal extension (6, 12), and the recently described human PRK1 and PRK2 sequences predicted from the corresponding cDNA clones (6, 9) (Fig. 3). The sequences of the 13 additional tryptic peptide sequences most closely matched (99% identity) sequences within the N-terminal noncatalytic domain region of human PRK2 (Fig. 3). Overall, the tryptic peptide sequences accounted for a total of 151 residues and were distributed throughout the predicted PRK2 sequence (9), including regions unique to the PRK2 sequence (peptides T12 and T13, Fig. 3).

To further investigate the relationship between PAK-2 and the PRKs, oligonucleotide primers based on conserved PRKs and selected PAK-2 tryptic peptide sequences, were used to screen total RNA fractions from rat tissues and rat myeloma by RT-PCR amplification. Intense PAK-2/PRK2-probe-specific PCR products were detected with the RNA of the rat myeloma Y3 cell line (20); only relatively trace quantities of the same products were detected with liver and none with other rat tissues tested (not illustrated). The composite rat myeloma cDNA sequence derived from overlapping PCR-generated fragments encoded an amino acid sequence exhibiting 93% sequence identity with the human PRK2-(1–984) (including C-terminal PCR primer sequence), and 98% identity with the sequences of the 17 tryptic peptides generated from PAK-2 (Fig. 3). Surprisingly, many of the sequence variations were clustered within the region adjacent to the catalytic domain, particularly the PRK2-(615–639) sequence (16 out of 25 amino acid differences, Fig. 3). The N-terminal peptide sequences closely matched (95.2% identity in region of overlap) a partial mouse sequence corresponding to human PRK2-(11–158) (Fig. 3). Given the alignment of closely matching rat PAK-2-derived tryptic peptides numbered in order T1-T17 (peptides T4, T5, and T12, resulted from non-tryptic-like activity) the asterisk against T5 indicates a composite sequence based on the sequences identified for three tryptic variants. The boxed-in regions correspond to the catalytic domain consensus sequence (12) (see footnote 3). The N- and C-terminal sequences of rat PAK-2/PRK2 encased in dark background (white letters) correspond to the most terminal PRK2 sequences used successfully for PCR primers.

Catalytic Properties of PAK-2—To assess the extent of the similarities in the catalytic properties of the structurally similar rat PRKs, detailed comparisons were made between the catalytic properties of PAK-2/PRK2 and the previously determined properties of liver PAK-1/PKN (5, 13, 16). The investigations focussed initially on the trypsin-activated form of the enzyme which, by analogy with liver PAK-1/PKN (5, 13), was considered likely to reflect the fully activated form, although it was recognized that the physiological significance of proteolysis as an activation mechanism (5, 19) is doubtful. The best substrates for trypsin-activated PAK-2/PRK2 were the ribosomal S6-(229–239) peptide (5, 17), with the lowest $K_m$ of 0.13 mM and a favorable $V_{max}$ of 2.7 mmol/min/mg, and the skeletal muscle glycogen synthase GS-(1–12) peptide analogue (17), with the highest $V_{max}$ of 8.8 mmol/min/mg and favorable $K_m$ of 3.2 mM. In the absence of trypsin activation, the PAK-2/PRK2 holoenzyme still had appreciable activity with the S6 peptide, although exhibiting a lower $V_{max}$ of 0.4 mmol/min/mg and higher $K_m$ (2.4

**Fig. 3. Primary structural analysis of rat liver PAK-2/PRK2.** The PAK-2/PRK2 amino acid sequence (single-letter code) is predicted from the nucleotide sequence of overlapping PCR products, generated from rat myeloma Y3 total RNA by RT-PCR, as described under “Experimental Procedures.” The sequence is aligned with the complete human PRK2 sequence (9), a partial mouse PRK2-like sequence (see footnote 4) and the rat PAK-1/PKN catalytic domain sequence (12), the amino acid residue numbering is based on the human PRK2 sequence. Underlined regions (with peptides T4, T5, T11, and T13, gaps indicate no residue identified) match the sequences of PAK-2/PRK2-derived tryptic peptides numbered in order T1-T17 (peptides T4, T5, and T12, resulted from non-tryptic-like activity). The asterisk against T5 indicates a composite sequence based on the sequences identified for three tryptic variants. The boxed-in regions correspond to the catalytic domain consensus sequence (12) (see footnote 3). The N- and C-terminal sequences of rat PAK-2/PRK2 encased in dark background (white letters) correspond to the most terminal PRK2 sequences used successfully for PCR primers.
PAK-2/PRK2 was assayed with synthetic peptides and protein substrates as described under "Experimental Procedures." Relative phosphotransferase activity is expressed as a percentage of that obtained with S6-(229–239) peptide substrate for trypsin-activated PAK-2/PRK2 (mean of duplicate determinations). The absolute activity (100%) for the trypsin-activated enzyme with the S6-(229–239) substrate was 30 pmol/min. The results are representative of two independent experiments.

| Substrate                                  | PAK-2/PRK2 relative phosphotransferase activity |
|--------------------------------------------|-----------------------------------------------|
|                                            | + Trypsin | - Trypsin |
| S6-(229–239)                               | 100       | 15        |
| GS-(1–12)                                  | 320       | 78        |
| EGFR-(650–658)                             | 30        | 1         |
| Prostate sulfate                           | 54        | 58        |
| Myelin basic protein                       | 4.7       | 1.7       |
| Histone IIIS                               | 2.0       | 0.25      |
| Histone IIA                                | 2.1       | 0.64      |
| Phosvitin                                  | 0.07      | 0.06      |
| Casein                                     | 0         | 0         |

Important: These kinetic properties were appreciably more favorable than for liver PAK-1/PKN, which were characterized by 10–40-fold higher $K_m$ values for the corresponding substrates (5).

As with PAK-1/PKN (5), PAK-2/PRK2 exhibited a preference for highly basic protein substrates, particularly nucleic acid binding proteins (Table I). The overall phosphotransferase activity was appreciably less (by 20–50-fold) with protein (with the exception of protamine, see below) compared with S6 peptide substrate, either with or without trypsin activation (Table I). The extent of activation varied with different protein substrates, ranging from 8 fold activation with type IIIS histone (histone H1) to relatively small activations with myelin basic protein and type IIA mixed histone (Table I). The protamine sulfate substrate was unique in that it exhibited very favorable kinetics of phosphotransferase ($K_m$ 0.2 μM; $V_{max}$ 1.3 μmol/min/mg) either with or without trypsin “activation,” indicating that the highly basic protamine overcame the intramolecular inhibition characteristic of PAK-2/PRK2. PAK-1/PKN and the PKCs exhibit a similar property (e.g. see Refs. 5, 25, and 26), except that the functional catalytic fragment of liver PAK-1/PKN generated by trypsin cleavage is relatively inactive toward the protamine substrate (5).

The liver PAKs can also be distinguished by their sensitivities toward various inhibitors, particularly the PKC-α (19–31) pseudosubstrate peptide (5, 27) which strongly inhibited PAK-2/PRK2 (IC$_{50}$ 1 μM), but not PAK-1/PKN (IC$_{50}$ 800 μM, see Morrice et al. (5). The S6-(229–239) peptide kinase activity of PAK-2/PRK2 was also more sensitive (3–8-fold) than liver PAK-1/PKN (5) to inhibition by poly-L-arginine and poly-L-lysine, with IC$_{50}$ values obtained for PAK-2/PRK2 of 0.1 and 0.4 μg/ml, respectively, whereas PAK-2/PRK2 was less sensitive to inhibition by NaF, with an IC$_{50}$ of 85 μM compared with 30 μM for PAK-1/PKN (5).

**Lipid Sensitivity of PAK-2/PRK2**—The S6-(229–239) peptide kinase activity of PAK-2/PRK2 was unresponsive to phosphatidylinerine, either in the presence or absence of diacylglycerol and Ca$^{2+}$ ion (not illustrated). Other acidic phospholipids and unsaturated fatty acids, including oleic acid, previously shown to be effective activators (4–5-fold) of liver PAK-1/PKN, in vitro (5, 13), only marginally increased (up to 60%) the S6-(229–239) peptide kinase activity of PAK-2/PRK2 (not illustrated). Phosphorylation of myelin basic protein and type IIIS histone also appeared to be unresponsive to cardiolipin (CL) (not illustrated). However, CL stimulated appreciably and selectively the phosphorylation of a major discrete polypeptide in

#### TABLE I

| Substrate      | Autophosphorylation (%) |
|----------------|-------------------------|
|                | 0 | 150 | 300 | 450 | 600 | 750 |
| Control        | - | - | - | - | - | - |
| CL             | - | - | - | - | - | - |
| PS             | - | - | - | - | - | - |
| PI             | - | - | - | - | - | - |
| MD             | - | - | - | - | - | - |
| PC             | - | - | - | - | - | - |
| PE             | - | - | - | - | - | - |
| PA             | - | - | - | - | - | - |
| Linoleic acid  | - | - | - | - | - | - |
| Linolenic acid | - | - | - | - | - | - |
| Arachidonic acid| - | - | - | - | - | - |

**Fig. 4. Effects of phospholipids and fatty acids on autophosphorylation of PAK-2/PRK2.** Autophosphorylation of purified PAK-2/PRK2 (45-min reaction) was assayed in the presence of various lipids or fatty acids at concentrations of 25 μg/ml or 50 μM, respectively, under the conditions described in "Experimental Procedures." Samples were analyzed by SDS-PAGE and autoradiographed as in Fig. 2B and, subsequently, the 32P radioactivity in excised gel pieces containing the 130-kDa PAK-2/PRK2 polypeptide was determined by liquid scintillation counting. Autophosphorylation (32P incorporation) in response to lipid activation of the enzyme is expressed as a percentage (mean of duplicate determinations) of the control (i.e. PAK-2/PRK2 autophosphorylation without additions). As an example, absolute values for autophosphorylation of PAK-2/PRK2 in the absence and presence of cardiolipin were 1.5 and 10.5 mol of phosphate incorporated/mol of enzyme. Results are representative of two independent experiments. PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; MD, 1,2-dimyristoyl-rlac-glycerol.

The lipid activatability of PAK-2/PRK2 was even more evident with PAK-2/PRK2 autophosphorylation, which was stimulated by the acidic phospholipids, CL (7 fold) and to a lesser extent phosphatidylinositol, phosphatidylserine, and phosphatic acid (1.5–3 fold) (Fig. 4). However, in comparison with liver PAK-1/PKN, the PAK-2/PRK2 isofrom was relatively less responsive to oleic acid and other unsaturated fatty acids (Fig. 4). As with PAK-1/PKN, PAK-2/PRK2 was unresponsive to the basic phospholipids phosphatidylethanolamine and phosphatidylcholine, as well as the diacylglycerol 1,2-dimyristoyl-rac-glycerol (Fig. 4). The most potent activator, CL, stimulated (~7-fold) both the rate and extent (maximum stoichiometry of 10.5 mol of phosphate/mol of kinase) of PAK-2/PRK2 autophosphorylation (not illustrated), with an EC$_{50}$ of 11 μg/ml, which was lower than that for histone H2B, but still higher than the value of 1 μg/ml reported previously for liver PAK-1/PKN autophosphorylation (13).

**Concluding Comments**—The discovery and characterization of a rat counterpart of PRK2 in liver are important for several reasons. First, it establishes the presence of this PRK isofrom in a normal tissue, thus, suggesting that the failure (this study as well as in Palmer and Parker (9)) to detect a PRK2-like mRNA in such tissues is due to the low abundance of the...
mRNA and/or secondary structure interference. Second, the detection of relatively intense PAK-2/PRK2 PCR products in PCR analyses of myeloma compared with liver RNA suggests a higher mRNA abundance, possibly linked with the cancerous state of myelomas (9). Thirdly, the biochemical properties of the naturally occurring PAK-2/PRK2 enzyme provide an experimental basis for investigating its physiological function. For example, the cytosolic location of a low activity form of liver PAK-2/PRK2 and its activatability by acidic phospholipids indicate that cellular activation and any intracellular targeting may involve signal transduction mechanisms directed toward the cytosolic enzyme.

Notwithstanding many similarities between rat liver PAK-2/PRK2 and PAK-1/PRK1, the differences in their catalytic properties suggest functionally distinct roles. The retention of most of the distinguishing properties by the catalytic fragments of the rat PAKs indicates that the 39 amino acid variations within their respective catalytic domains, which are mainly concentrated in the N-terminal region (Fig. 3), determine these functional differences. The conservation of 33 of these sequence differences between the catalytic domains of the individual rat PAK-1/PRK1 and PAK-2/PRK2 isoforms (Fig. 3) (6, 12) and the corresponding human PRK1/PKN and PRK2 (9) isoforms suggests that their distinct catalytic properties are also likely to be highly conserved.

The complex properties of the negatively regulatory domains of the PAKs/PKN/PRKs suggest that a number of regulatory mechanisms might be available to the enzyme, in vivo, including interactions with Rho-type proteins (14, 15) and lipid second messengers and direct phosphorylation events (12). Whether PAK-2/PRK2 also interacts with RhoA or related proteins remains to be determined. There is appreciable sequence variation (58% identity) within the region of human PKN/PRK1 (PKN-34–103) implicated in RhoA binding (14, 15) and the corresponding region of PAK-2/PRK2 (9) (Fig. 3), hence the possibility of a different specificity for interacting proteins cannot be discounted. The specific nature of any physiological lipid activators of the PAKs/PKN/PRKs also remains to be determined. Presumably, CL mimics the action of a physiological mediator since it is confined to mitochondria in normal mammalian cells and, therefore, is not directly accessible to the cytosolic PAK-2/PRK2 enzyme. An additional complexity is the different pattern of lipid effects for the two liver PAKs, suggesting subtle differences in the functioning of their regulatory domains. Given the various mechanisms available for regulating PAK-2/PRK2-type enzymes and the potential for interactions between the different regulatory events, it will be important to take into account the biochemical properties of the naturally occurring form of the liver enzyme in the design of future investigations of their cellular roles.

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