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Identity between the Ca^{2+}-independent Phospholipase A_2 Enzymes from $P388D_1$ Macrophages and Chinese Hamster Ovary Cells*

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A novel Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) has recently been purified and characterized from $P388D_1$ macrophages (Ackermann, E. J., Kempner, E. S., and Dennis, E. A. (1994) *J. Biol. Chem.* 269, 9227–9233). This enzyme appears to play a key role in regulating basal phospholipid remodeling reactions. Also an $iPLA_2$ from Chinese hamster ovary (CHO) cells has been purified, molecularly cloned, and expressed (Tang, J., Kriz, R., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) *J. Biol. Chem.* 272, 8567–8575). We report herein that the cloned CHO $iPLA_2$ is equivalent to the mouse enzyme purified from $P388D_1$ cells. Polymerase chain reaction amplification of cDNA fragments from $P388D_1$ cells using primers based on the CHO $iPLA_2$ sequence, revealed a high degree of homology between the mouse and hamster enzymes at both the nucleotide and amino acid levels (92 and 95%, respectively). Identity between the two proteins was further demonstrated by using immunochemical, pharmacological, and biochemical approaches. Thus, an antiserum generated against the CHO enzyme recognized the $P388D_1$ cell enzyme and gave similar molecular masses (about 83 kDa) for the two enzymes under the same experimental conditions. Further, the CHO enzyme has exactly the same sensitivity to inhibition by a variety of compounds previously shown to inhibit the $P388D_1$ enzyme, including bromoenol lactone, palmitoyl trifluoromethyl ketone, and methyl arachidonyl fluorophosphonate. Additionally, covalent modification of the CHO enzyme by [^{3}H]bromoenol lactone is dependent on active enzyme as is the $P388D_1$ $iPLA_2$. Finally, both enzymes have the same specific activities under identical experimental conditions.

Phospholipase A_2 (PLA_2)[1] comprises a superfamily of enzymes that regulate phospholipid metabolism and generate bioactive lipid mediators such as the eicosanoids and platelet-activating factor (for review see Ref. 1). Since the PLA_2s have been implicated in a number of tissue dysfunctions ranging from inflammation to ischemia, much attention has been devoted to the study of the mechanism of action and biochemical characteristics of these enzymes. Depending on their site of action, the PLA_2s can be subdivided into two types: the extracellular, secreted enzymes and the intracellular, cytoplasmic enzymes (1). Among the latter, two groups of enzymes can be considered, namely the group IV, Ca_{2+}-dependent cytosolic PLA_2, and the Ca^{2+}-independent PLA_2 ($iPLA_2$).

In contrast to the group I–IV PLA_2s, the Ca^{2+}-independent PLA_2s ($iPLA_2$) have been poorly studied, since most of them are labile, constitute only a minor fraction of the total cellular protein, and have lower specific activities. The $iPLA_2$s have been grouped into three main categories based on their biochemical and localization characteristics: lysosomal, brush-border membranes, and intracellular (for review, see Ref. 2). The lysosomal and brush-border membrane $iPLA_2$s appear to be conserved among the species where they have been identified. However, the intracellular $iPLA_2$ enzymes appear to represent a much more diverse and broad group of enzymes, whose relationship is not so evident. Only four intracellular $iPLA_2$s have been purified to homogeneity, namely a 40-kDa enzyme from myocardium (3), a 39-kDa enzyme from bovine brain (4), an 80-kDa enzyme from $P388D_1$ macrophages (5), and very recently a 28-kDa enzyme from rabbit kidney (6). The enzymes from myocardium and $P388D_1$ cells are both modulated by ATP and form oligomeric complexes of about 400 kDa. However, besides their very distinct molecular mass, they also differ significantly in substrate preference and detergent sensitivity (3, 5).

The first molecular cloning of an $iPLA_2$ from CHO cells is reported in the accompanying manuscript (7). Due to the role of $iPLA_2$ in $P388D_1$ cell metabolism (8), it was essential to determine whether or not the enzyme present in these cells was the same as that cloned by Tang *et al.* (7). Using a variety of techniques, we demonstrate herein that the enzyme from $P388D_1$ cells and the cloned enzyme from CHO cells is the same molecular entity expressed in different species. Thus, the availability of the macrophage $iPLA_2$ sequence should ensure rapid progress in understanding its physiological function.

EXPERIMENTAL PROCEDURES

Materials—1-Palmitoyl-2-[^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine was obtained from Amersham Corp. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti (Birmingham, AL). (*E*)-6-(bromomethylene)-tetrahydro-3-(1-[4-^{3}H]naphthalenyl)-2H-pyran-2-one ([^{3}H]BEL) was a generous gift of Drs. Randy H. Weiss and Philip Needleman (Monsanto Co., St. Louis, MO). Palmitoyl trifluoromethyl ketone ($PACOCF_3$) and BEL were synthesized in our laboratory as described previously (9). Methyl arachidonyl fluorophosphonate (MAFP) was purchased from Cayman Chemical Co. (Ann Arbor, MI).

$iPLA_2$ Assay—The $iPLA_2$ activity has been previously described (5). Briefly, 10–50 ng of purified $iPLA_2$ was assayed in a buffer consisting of 100 mM Hepes, 400 μM Triton X-100, 100 μM 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, 1-palmitoyl-2-[^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine (200,000 cpm), 5 mM EDTA, and 0.1 mM ATP (pH 7.5). The

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U88624.

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[1] The abbreviations used are: PLA_2, phospholipase A_2; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; BEL, bromoenol lactone; CHO, Chinese hamster ovary; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); MAFP, methyl arachidonyl fluorophosphonate; $PACOCF_3$, palmitoyl trifluoromethyl ketone; PCR, polymerase chain reaction.

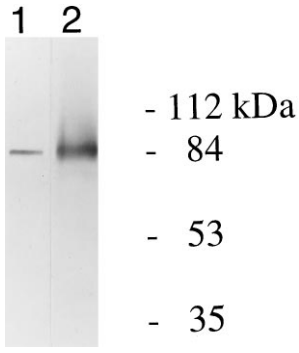

FIG. 1. **Immunoblot of the iPLA$_2$ from P388D$_1$ and CHO cells.** Purified iPLA$_2$ from CHO cells (*lane 1*) and P388D$_1$ cells (*lane 2*) was subjected to 10% SDS-polyacrylamide gel electrophoresis, transferred to a membrane, and probed with an antiserum to CHO iPLA$_2$. Bands were detected by chemiluminiscence (ECL).

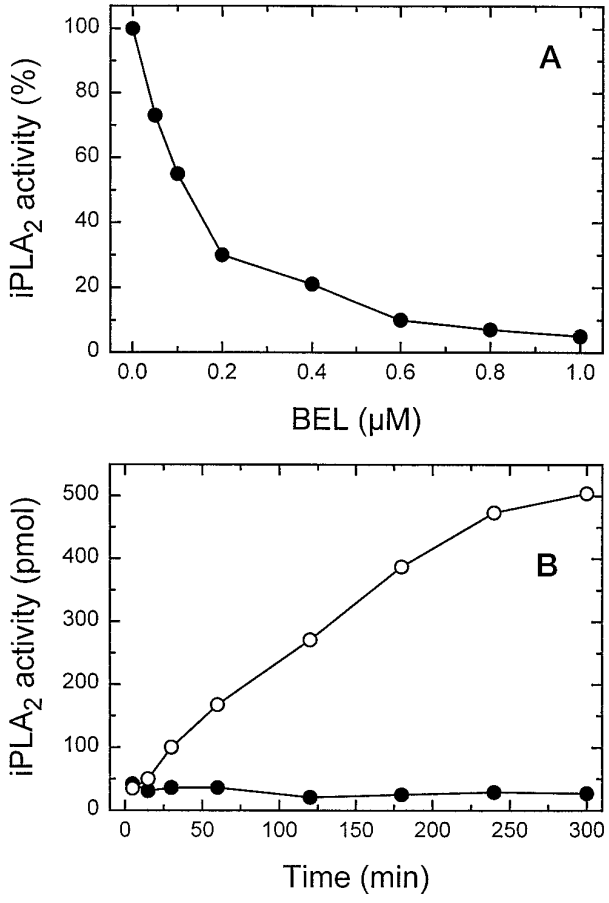

FIG. 2. **Effect of BEL on CHO cell iPLA$_2$ activity.** *A*, partially purified recombinant iPLA$_2$ was incubated in the presence of BEL for 5 min at 37 °C; the remaining activity was assayed using mixed micelles composed of 500 μM Triton X-100 and 100 μM 1-palmitoyl-2-arachidonoyl-glycerophosphocholine. *B*, time course of iPLA$_2$ activity that was preincubated with (*filled circles*) or without (*open circles*) of 5 μM BEL and then exhaustively dialyzed for 18 h.

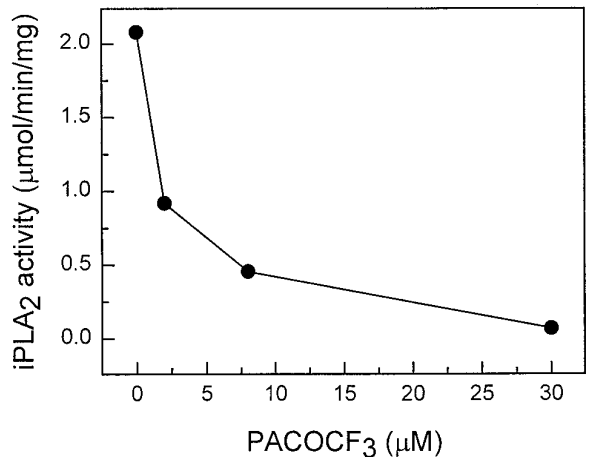

FIG. 3. **Effect of PACOCF$_3$ on CHO cell iPLA$_2$ activity.** Purified CHO cell iPLA$_2$ was assayed as described under "Experimental Procedures" in the presence of the indicated concentrations of PACOCF$_3$.

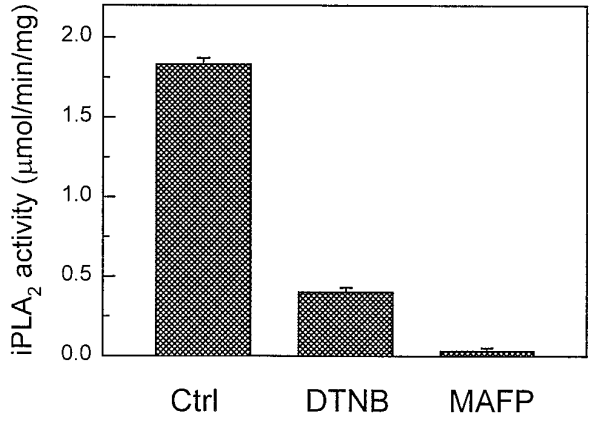

FIG. 4. **Effect of DTNB and MAFP on CHO cell iPLA$_2$ activity.** CHO cell iPLA$_2$ was preincubated with 1 mM DTNB or 20 μM MAFP for 5 min at 40 °C, followed by dilution into assay buffer and quantification of the activity remaining.

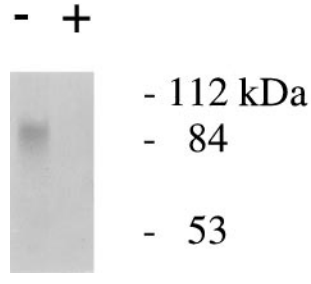

FIG. 5. **[^{3}H]BEL labeling of CHO cell iPLA$_2$.** CHO cell iPLA$_2$ was incubated with (+) or without (−) 20 μM MAFP for 5 min at 40 °C, as indicated. Afterward, the protein was incubated with [^{3}H]BEL for 30 min at 40 °C. After washing out the excess of [^{3}H]BEL, proteins were separated by SDS-polyacrylamide gel electrophoresis on a 10% gel.

mixture was incubated at 40 °C for 30 min with shaking, the reaction was stopped by adding 2.5 ml of Dole reagent (2-propanol, heptane, 0.5 M H_2SO_4; 400:100:20 (v/v/v)), and products were processed by a modified Dole extraction procedure (10). When the irreversible inhibitor BEL, MAFP, or 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was used in the assays, the enzyme was preincubated with the inhibitor for 5 min at 40 °C. The remainder of enzyme activity was assayed by adding the substrate mixture. When the reversible inhibitor PACOCF$_3$ was used, it was added directly to the assay mixture.

Immunoblot—Purified proteins were analyzed by a 10% SDS-polyacrylamide gel electrophoresis (Novex), transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore Corp.). Nonspecific binding was blocked by incubating the membranes with a buffer consisting of 5% nonfat milk, 10 mM Tris-HCl, pH 8, 1 mM EDTA, 150 mM NaCl, and 0.1% Triton X-100 for 60 min. Membranes were incubated with the antiserum generated against the CHO iPLA$_2$ (1:200 into the blocking buffer) for 30 min and then treated with horseradish peroxidase-conjugated protein A (Amersham). Bands were detected by enhanced chemiluminiscence (ECL; Amersham).

[^{3}H]BEL Labeling—100 ng of the CHO or P388D$_1$ cell iPLA$_2$ was incubated with 7 μM [^{3}H]BEL for 30 min at 40 °C. Excess unreacted [^{3}H]BEL was washed out eight times with H_2O using a Centricon 10 (Amicon Inc.). Samples were then analyzed by 10% SDS-polyacrylamide gel electrophoresis (Novex). Gels were fixed with 40% methanol, 10%

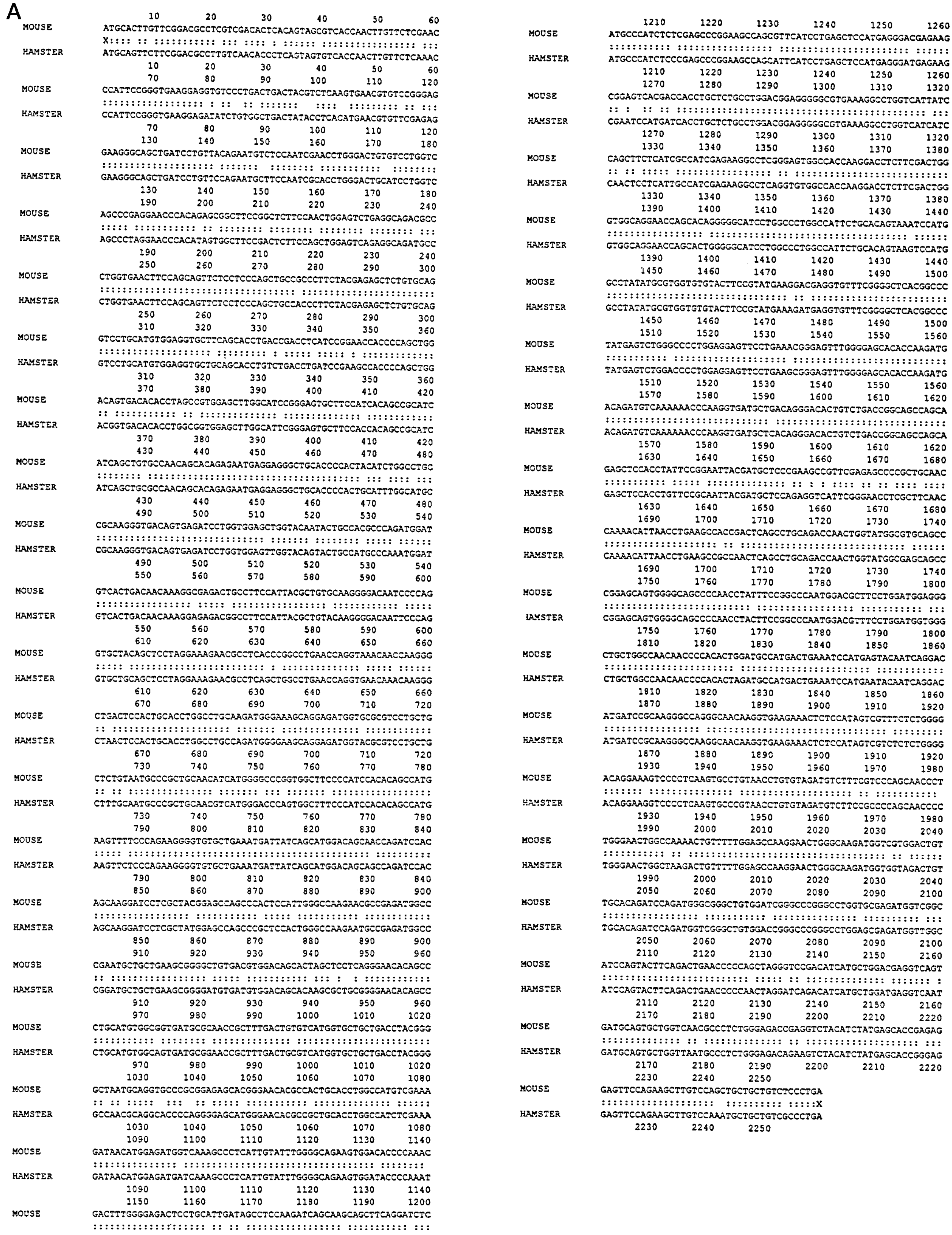

FIG. 6. **Comparison between the nucleotide (*A*) and amino acid (*B*) sequences of mouse and hamster $iPLA_2$.** A *colon* denotes nucleotides or amino acids that are identical; a *dot* denotes a conservative amino acid change.

acetic acid and incubated for 30 min with a fluorographic reagent (Amplify, Amersham). Bands were visualized by autoradiography (9). When MAFP was used to prevent [^{3}H]BEL binding, purified enzyme was preincubated with 20 μM MAFP for 15 min at 40 °C prior to the addition of [^{3}H]BEL.

$P388D_1$ $iPLA_2$ Sequencing—Total RNA from $P388D_1$ cells was iso-

B

```
                10        20        30        40        50        60
MOUSE.PRO       MHLFGRLVDTHSSVTNLFSNPFRVKEVSLTDYVSSERVREEGQLILLQNVSNRTWDCVLV
                :..:::::.: :::::::::::::::.:...::.: :::::::::::.::.:::::::.::
HAMSTER.PRO     MQFFGRLVNTLSSVTNLFSNPFRVKEISVADYTSHERVREEGQLILFQNASNRTWDCILV
                10        20        30        40        50        60
                70        80        90       100       110       120
MOUSE.PRO       SPRNPQSGFRLFQLESEADALVNFQQFSSQLPPFYESSVQVLHVEVLQHLTDLIRNHPSW
                ::::::.:::::::::::::::::::::::::::::::::::::::::.::::.::::
HAMSTER.PRO     SPRNPHSGFRLFQLESEADALVNFQQFSSQLPPFYESSVQVLHVEVLQHLSDLIRSHPSW
                70        80        90       100       110       120
               130       140       150       160       170       180
MOUSE.PRO       TVTHLAVELGIRECFHHSRIISCANSTENEEGCTPLHLACRKGDSEILVELVQYCHAQMD
                ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HAMSTER.PRO     TVTHLAVELGIRECFHHSRIISCANSTENEEGCTPLHLACRKGDSEILVELVQYCHAQMD
               130       140       150       160       170       180
               190       200       210       220       230       240
MOUSE.PRO       VTDNKGETAFHYAVQGDNPQVLQLLGKNASPGLNQVNNQGLTPLHLACKMGKQEMVRVLL
                ::::::::::::::::::.:::::::::::.:::::::.:::::::::::.::::::::::
HAMSTER.PRO     VTDNKGETAFHYAVQGDNSQVLQLLGKNASAGLNQVNKQGLTPLHLACQMGKQEMVRVLL
               190       200       210       220       230       240
               250       260       270       280       290       300
MOUSE.PRO       LCNARCNIMGPGGFPIHTAMKFSQKGCAEMIISMDSNQIHSKDPRYGASPLHWAKNAEMA
                :::::::.:::.:::::::::::::::::::::::.:::::::::::::::::::::::::
HAMSTER.PRO     LCNARCNVMGPSGFPIHTAMKFSQKGCAEMIISMDSSQIHSKDPRYGASPLHWAKNAEMA
               250       260       270       280       290       300
               310       320       330       340       350       360
MOUSE.PRO       RMLLKRGCDVDSTSSSGNTALHVAVMRNRFDCVMVLLTYGANAGARGEHGNTPLHLAMSK
                ::::::::::::::..:::::::::::::::::::::::::::::..:::::::::::::
HAMSTER.PRO     RMLLKRGCDVDSTSAAGNTALHVAVMRNRFDCVMVLLTYGANAGTPGEHGNTPLHLAISK
               310       320       330       340       350       360
               370       380       390       400       410       420
MOUSE.PRO       DNMEMVKALIVFGAEVDTPNDFGETPALIASKISKQLQDLMPISRARKPAFILSSMRDEK
                :::::.:::::::::::::::::::..:::::::::::::::::::::::::::::::::
HAMSTER.PRO     DNMEMIKALIVFGAEVDTPNDFGETPAFMASKISKQLQDLMPISRARKPAFILSSMRDEK
               370       380       390       400       410       420
               430       440       450       460       470       480
MOUSE.PRO       RSHDHLLCLDGGGVKGLVIIQLLIAIEKASGVATKDLFDWVAGTSTGGILALAILHSKSM
                : ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HAMSTER.PRO     RIHDHLLCLDGGGVKGLVIIQLLIAIEKASGVATKDLFDWVAGTSTGGILALAILHSKSM
               430       440       450       460       470       480
               490       500       510       520       530       540
MOUSE.PRO       AYMRGVYFRMKDEVFRGSRPYESGPLEEFLKREFGEHTKMTDVKKPKVMLTGTLSDRQPA
                ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HAMSTER.PRO     AYMRGVYFRMKDEVFRGSRPYESGPLEEFLKREFGEHTKMTDVKKPKVMLTGTLSDRQPA
               490       500       510       520       530       540
               550       560       570       580       590       600
MOUSE.PRO       ELHLFRNYDAPEAVREPRCNQNINLKPPTQPADQLVWRAARSSGAAPTYFRPNGRFLDGG
                :::::::::::..::::: ::::::::::::::::::::::::::::::::::::::::::
HAMSTER.PRO     ELHLFRNYDAPEVIREPRFNQNINLKPPTQPADQLVWRAARSSGAAPTYFRPNGRFLDGG
               550       560       570       580       590       600
               610       620       630       640       650       660
MOUSE.PRO       LLANNPTLDAMTEIHEYNQDMIRKGQGNKVKKLSIVVSLGTGKSPQVPVTCVDVFRPSNP
                ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
HAMSTER.PRO     LLANNPTLDAMTEIHEYNQDMIRKGQGNKVKKLSIVVSLGTGRSPQVPVTCVDVFRPSNP
               610       620       630       640       650       660
               670       680       690       700       710       720
MOUSE.PRO       WELAKTVFGAKELGKMVVDCCTDPDGRAVDRARAWCEMVGIQYFRLNPQLGSDIMLDEVS
                ::::::::::::::::::::::::::::::::::::.:::::::::::::::::::::::.
HAMSTER.PRO     WELAKTVFGAKELGKMVVDCCTDPDGRAVDRARAWSEMVGIQYFRLNPQLGSDIMLDEVN
               670       680       690       700       710       720
               730       740       750
MOUSE.PRO       DAVLVNALWETEVYIYEHREEFQKLVQLLLSP
                ::::::::::::::::::::::::::.::::
HAMSTER.PRO     DAVLVNALWETEVYIYEHREEFQKLVQMLLSP
               730       740       750
```

FIG. 6—*continued*

lated using the guanidinium isothiocyanate/phenol chloroform extraction method (Stratagene). Polyadenylated mRNA was purified by affinity chromatography using an oligo(dT)-cellulose column (Stratagene). The mRNA was converted to cDNA using the Stratagene reverse transcriptase-PCR kit. Amplification of specific cDNA fragments was achieved by using recombinant *Pfu* polymerase (Stratagene). Primers were based on the CHO $iPLA_2$ sequence (Ref. 7; GenBank™ accession number I15470). The 5′-primer used (AGG ATG CAG TTC TTC GGA CGC C) included the translation initiation codon ATG. The 3′-primer was a reverse primer (CAG TTG ATG GAG CCA GTT GTC C) situated 223 bases after the termination codon TGA. To determine the sequence of the 5′-end (primer region), we performed a standard rapid amplification of cDNA ends-PCR (9). Briefly, cDNA was amplified by PCR using the mouse reverse primer TTC CTA GGA GCT GTA GCA CCT G, which is equivalent to nucleotides 598–619 in the hamster sequence. After attaching a poly(A) tail to the 5′-end of the PCR product obtained, amplification of this product was accomplished by PCR using a dT(18) primer and a mouse reverse primer, ACT CCA GTT GGA AGA GCC GGA A, which is equivalent to nucleotides 205–226 in the hamster sequence. A final round of amplification was performed using the mouse reverse primer TTC CTC CCG GAC ACG TTC ACT T (equivalent to nucleotides 101–123 in the hamster sequence). PCR conditions were as follows: denaturing, 95 °C for 30 s; annealing, 55 °C for 45 s; extension, 72 °C for 120 s; 40 cycles. A last extension step lasted 10 min. PCR products were purified by agarose gel electrophoresis and sequenced by automatic DNA cycle sequencing (Applied Biosystems 373 automated DNA sequencer, Perkin-Elmer).

RESULTS AND DISCUSSION

The intracellular $iPLA_2$ from $P388D_1$ cells was purified and characterized in our laboratory as an approximately 80-kDa protein (5). The $iPLA_2$ purified by Tang *et al.* (7) from CHO cells and the protein expressed by the cDNA have a molecular size of 84.5 kDa. Besides this similarity, the purification strategy used by Tang *et al.* (7) contains some analogous steps to those used for the macrophage $iPLA_2$ (5), and the enzymatic profile of the CHO enzyme is comparable. Therefore, we hypothesized that both proteins would share a high degree of similarity if not identity. We began to address this hypothesis by taking advantage of the polyclonal antibody generated by Tang *et al.* (7) against the C-terminal portion of the CHO protein. The antiserum recognized pure $iPLA_2$ from $P388D_1$ cells, as shown by immunoblot (Fig. 1, *lane 2*). Purified CHO $iPLA_2$ was run in parallel as a control and showed identical mobility, focusing both proteins in the 80–85-kDa molecular mass range (Fig. 1, *lane 1*).

To further establish a similarity between the two proteins, a series of inhibition studies were conducted. We have previously shown that the $iPLA_2$ from $P388D_1$ cells is potently and irreversibly inhibited by bromoenol lactone (BEL). After a preincubation time of 5 min at 40 °C, BEL inhibits pure $iPLA_2$ with an IC_{50} of about 60 nM (10). Likewise, $iPLA_2$ from CHO cells was inhibited in a dose-dependent manner by BEL with an IC_{50} of 120 nM (Fig. 2*A*). This inhibition was irreversible even after exhaustive dialysis of treated enzyme compared with control (Fig. 2*B*). Also, $P388D_1$ $iPLA_2$ is reversibly inhibited by $PACOCF_3$ (IC_{50} = 4 μM), and again CHO $iPLA_2$ was inhibited by the same reagent with an IC_{50} of 3 μM (Fig. 3).

Since BEL is an irreversible inhibitor, it was possible to label the macrophage $iPLA_2$ by incubation with [^{3}H]BEL (10). However, labeling with BEL required the presence of active enzyme, since the inhibitory agent is generated *in situ* from BEL by the hydrolytic action of the enzyme on the lactone ring (10). Indeed, in previous studies with the $P388D_1$ $iPLA_2$, DTNB prevented labeling of the enzyme with [^{3}H]BEL (10). Although DTNB also inhibits the CHO $iPLA_2$ (Fig. 4), we have employed herein the irreversible inhibitor MAFP (11), because it is a much more specific reagent than DTNB. MAFP (20 μM) completely inactivated the CHO $iPLA_2$ (Fig. 4) and prevented labeling of the enzyme with [^{3}H]BEL (Fig. 5). Moreover, in the absence of MAFP treatment, autoradiographic analysis of [^{3}H]BEL-treated enzyme revealed a single spot at about 85 kDa. This is the same molecular size as that obtained for the $P388D_1$ $iPLA_2$ utilizing a similar strategy (10) as well as that found by immunoblot (Fig. 1).

The nucleotide sequence of the $P388D_1$ $iPLA_2$ was obtained by analyzing mRNA from these cells by PCR using primers based on the CHO $iPLA_2$ sequence (Fig. 6). Comparison of the nucleotide sequence of the two proteins revealed a 92% sequence homology. Most of the changes of the nucleotide sequence occurred at the third nucleotide of the codon, reflecting the 95% identity at the amino acid level between two closely related species. These results further support the notion that the hamster $iPLA_2$ cloned by Tang *et al.* (7) is the species equivalent to the murine enzyme purified and characterized by us.

Our previous studies on the $P388D_1$ cell $iPLA_2$ have suggested that this enzyme may play an important role in regulating fatty acid remodeling reactions in the cells (8, 12), and a similar role has subsequently been attributed to the $iPLA_2$ activity present in rat pancreatic islets (13). This implies that the $iPLA_2$ enzyme might ultimately regulate several key as-

pects of cell physiology, such as new membrane synthesis that allows cell proliferation, or fatty acid exchange within phospholipids that allows adaptive homeostatic changes. Although the discovery by Gross and co-workers (14) that some $iPLA_2$s are potently and selectively inhibited by BEL has accelerated research on this enzyme, study of the $iPLA_2$ is hampered by the fact that it is extremely difficult to obtain sufficient amounts of pure protein for biochemical and sequence analysis.

We have presented evidence herein that the enzyme purified and cloned by Tang *et al.* (7) is the equivalent in hamster to the enzyme we identified (15), purified (5), characterized (5, 10, 11), and studied at the cellular level (8, 12) in mouse $P388D_1$ macrophages. By using a wide variety of approaches, we have found biochemical, immunological, pharmacological, and genetic similarities between the two proteins, strongly suggesting that the same molecular entity is expressed in different species. There is, however, an apparent difference between the two enzymes concerning ATP sensitivity. Tang *et al.* (7) failed to observe any ATP effect on a partially purified preparation of CHO $iPLA_2$. We have confirmed this finding using the assay system employed for the $P388D_1$ $iPLA_2$. We have previously reported on the apparent activation of $P388D_1$ $iPLA_2$ by ATP (5). The effect was shown to depend on the presence of Triton X-100 in the assay system. This, along with the fact that several other nucleotides manifested the same effect (*i.e.* ADP, UTP, GTP) raised the possibility that it might not be relevant as a regulatory mechanism. We have new data with $P388D_1$ $iPLA_2$ showing that, rather than stimulating the $iPLA_2$, the ATP stabilizes the enzyme and protects it from denaturation; hence, higher activity is found in the presence than in the absence of ATP.[2] Considering the fact that the two proteins have slightly different amino acid sequences and purification schemes, it is possible that the CHO enzyme lacks the region with which ATP interacts. Alternatively, it is possible that the CHO enzyme is more resistant to denaturation than the $P388D_1$ enzyme.

The availability of the $iPLA_2$ cDNA and protein sequence of the 80-kDa $iPLA_2$, which is now classified as a Group VI PLA_2 (16), will allow new experimental avenues to be explored in defining the role(s) of $iPLA_2$ in phospholipid metabolism and cellular function.

[2] Y. C. Lio and E. A. Dennis, manuscript in preparation.

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REFERENCES

1. Dennis, E. A. (1994) *J. Biol. Chem.* **269,** 13057–13060
2. Ackermann, E. J., and Dennis, E. A. (1995) *Biochim. Biophys. Acta* **1259,** 125–136
3. Hazen, S. L., and Gross, R. W. (1991) *Biochem. J.* **280,** 581–587
4. Hirashima, Y., Farooqui, A. A., Mills, J. S., and Horrocks, L. A. (1992) *J. Neurochem.* **59,** 708–714
5. Ackermann, E. J., Kempner, E. S., and Dennis, E. A. (1994) *J. Biol. Chem.* **269,** 9227–9233
6. Portilla, D., and Dai, G. (1996) *J. Biol. Chem.* **271,** 15451–15457
7. Tang, J., Kriz, R., Wolfman, N., Shaffer, M., Seehra, J., and Jones, S. S. (1997) *J. Biol. Chem.* **272,** 8567–8575
8. Balsinde, J., Bianco I., Ackermann, E. J., Conde-Frieboes, K., and Dennis, E. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 8527–8531
9. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 8998–9002
10. Ackermann, E. J., Conde-Frieboes, K., and Dennis, E. A. (1995) *J. Biol. Chem.* **270,** 445–450
11. Lio, Y-C., Reynolds, L. J., Balsinde, J., and Dennis, E. A. (1996) *Biochim. Biophys. Acta* **1302,** 55–60
12. Balsinde, J., and Dennis, E. A. (1996) *J. Biol. Chem.* **271,** 6758–6765
13. Ma, Z., Ramanadham, S., Corbett J. A., Bohrer, A., Gross R. W., McDaniel, M. L., and Turk J. (1996) *J. Biol. Chem.* **271,** 1029–1042
14. Hazen, S. L., Zupan, L. A., Weiss, R. H., Getman, D. P., and Gross, R. W. (1991) *J. Biol. Chem.* **266,** 7227–7232
15. Ross, M. I., Deems, R. A., Jesaitis, A. J., Dennis, E. A., and Ulevitch, R. J. (1985) *Arch. Biochem. Biophys.* **238,** 247–258
16. Dennis, E. A. (1997) *Trends Biochem. Sci.* **22,** 1–2

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