FEBS Letters 462 (1999) 442–446 FEBS 23008

Proteolytic cleavage of protein kinase Cμ upon induction of apoptosis in U937 cells

Identification of the cleavage site and characterization of the fragment

Sabine Häussermann[a], Walter Kittstein[a], Gabriele Rincke[a], Franz-Josef Johannes[b], Friedrich Marks[a], Michael Gschwendt[a,*]

[a] *German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg, Germany*
[b] *Fraunhofer Institute for Interfacial Chemistry and Biochemistry, Stuttgart, Germany*

Received 27 October 1999

Edited by Julio Celis

Abstract Treatment of U937 cells with various apoptosis-inducing agents, such as TNFα and β-D-arabinofuranosylcytosine (ara-C) alone or in combination with the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), bryostatin 1 or cycloheximide, causes proteolytic cleavage of protein kinase Cμ (PKCμ) between the regulatory and catalytic domain, generating a 62 kDa catalytic fragment of the kinase. The formation of this fragment is effectively suppressed by the caspase-3 inhibitor Z-DEVD-FMK. In accordance with these in vivo data, treatment of recombinant PKCμ with caspase-3 in vitro results also in the generation of a 62 kDa fragment (p62). Treatment of several aspartic acid to alanine mutants of PKCμ with caspase-3 resulted in an unexpected finding. PKCμ is not cleaved at one of the typical cleavage sites containing the motif DXXD but at the atypical site CQND^{378}/S^{379}. The respective fragment (amino acids 379–912) was expressed in bacteria as a GST fusion protein (GST-p62) and partially purified. In contrast to the intact kinase, the fragment does not respond to the activating cofactors TPA and phosphatidylserine and is thus unable to phosphorylate substrates effectively.

Key words: Protein kinase Cμ; Apoptosis; Caspase-3; Caspase-3 inhibitor; Cleavage site; Proteolytic fragment

1. Introduction

Human protein kinase C (PKC) μ (and its mouse homolog PKD) is a phospholipid-dependent, Ca^{2+}-independent serine/threonine protein kinase which, like the c- and n-type PKC isozymes (for reviews, see [1–5]), is stimulated by diacylglycerol (DAG) or phorbol esters but differs from the other PKCs in some structural and enzymatic properties [6–13]. PKCμ contains a pleckstrin homology (PH) domain, two unique amino-terminal hydrophobic domains and it lacks the typical pseudosubstrate motif. Moreover, PKCμ fails to phosphorylate several PKC substrates and to be inhibited by a PKC specific inhibitor. Also, the mechanism of activation of PKCμ appears to be different from that of the other DAG-activated PKCs. The cysteine-rich regions [14], the PH domain [15] and possibly an acidic domain [16,17] might play a role in the induction or suppression of PKCμ activity. In contrast to other PKCs [18–22], the role of phosphorylation of PKCμ for catalytic competence and activity of the kinase is not known. It has been suggested, however, that PKCμ is phosphorylated and activated by other members of the PKC family [23]. The cellular functions of PKCμ are not clear yet.

The activation of the caspase system is a critical event in apoptosis [24–26]. A number of signal transduction kinases, including several PKC isoenzymes, are subject to caspase-mediated breakdown. This results in the production of a catalytically active fragment of some kinases, whereas several other kinases are inactivated. For example, degradation upon induction of apoptosis of MEKK-1 [27–29], PKN [30], PKCδ [31–33], PKCε [33] and PKCθ [33] generates an active fragment each, whereas Raf-1 [34], Akt [34] and PKCζ [35] are inactivated during apoptosis. This is consistent with a model proposing the existence of pro-apoptotic and anti-apoptotic (pro-survival) kinases that are activated and inactivated, respectively, upon induction of apoptosis. Here, we show that, similarly to other PKCs, PKCμ undergoes specific proteolytic cleavage by caspases-3 upon induction of apoptosis in U937 cells, resulting in a 62 kDa catalytic fragment (p62).

2. Materials and methods

2.1. Reagents

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was supplied by Dr. E. Hecker, German Cancer Research Center (Heidelberg, Germany), and Gö6983 by Goedecke AG (Freiburg, Germany). Bryostatin 1 was provided by Dr. G.R. Pettit, State University of Arizona (Tempe, AZ, USA. Syntide 2 was synthesized by Dr. R. Pipkorn, German Cancer Research Center (Heidelberg, Germany). Recombinant PKCμ was expressed in a baculovirus-infected insect cell system as described previously [36].

Other materials were bought from the following companies: active human recombinant caspase-3 from Pharmingen (Hamburg, Germany); caspase-3 inhibitor Z-DEVD-FMK from Calbiochem (Schwalbach, Germany); bovine brain L-α-phosphatidylserine (PS), cycloheximide and β-D-arabinofuranosylcytosine (ara-C) from Sigma (Munich, Germany); [γ-^{32}P]ATP (specific activity, 5000 Ci/mmol) from Hartmann Analytic (Braunschweig, Germany); L-[^{35}S]methionine (specific activity, 1000 Ci/mmol) from Amersham Buchler

*Corresponding author. Fax: (49)-6221-42 4554.
E-mail: m.gschwendt@dkfz-heidelberg.de

Abbreviations: PKC, protein kinase C; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PS, L-α-phosphatidylserine; ara-C, β-D-arabinofuranosylcytosine; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis

PII: S0014-5793(99)01577-X

(Braunschweig, Germany); PKCμ specific polyclonal antibody sc-639 from Santa Cruz Biotechnology (Santa Cruz) and alkaline phosphatase-conjugated goat antibodies from Dianova (Hamburg, Germany); K252a from Fluka Chemie A.G. (Neu-Ulm, Germany); thrombin from Amersham Pharmacia (Freiburg, Germany); leupeptin and aprotinin from Roche Diagnostic (Mannheim, Germany).

2.2. Cell culture

Human U937 myeloid leukemia cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Cells were treated with various agents as indicated in the figure legends.

2.3. Preparation of cell extracts and immunoprecipitation

Cells were washed twice with phosphate-buffered saline (PBS) and stored at −75°C. Upon thawing, they were resuspended in lysis buffer (20 mM Tris-HCl, pH 7.5, 10 μg/ml aprotinin and 10 μg/ml leupeptin). The cell suspension was kept on ice for 30 min. Upon centrifugation at 100 000×*g* for 35 min, the supernatant (cell extract) was used for immunoprecipitation. The cell extract (1.5 mg protein) was incubated with 14 μg/ml of the anti-PKCμ antibody sc-639 in lysis buffer containing 150 mM NaCl (total volume 1 ml) at 4°C for 1.5 h and subsequently with 30 μl of protein-A-agarose at 4°C for 2 h. The precipitate was dissolved in 80 μl phosphorylation buffer and phosphorylated as described below under Section 2.8.

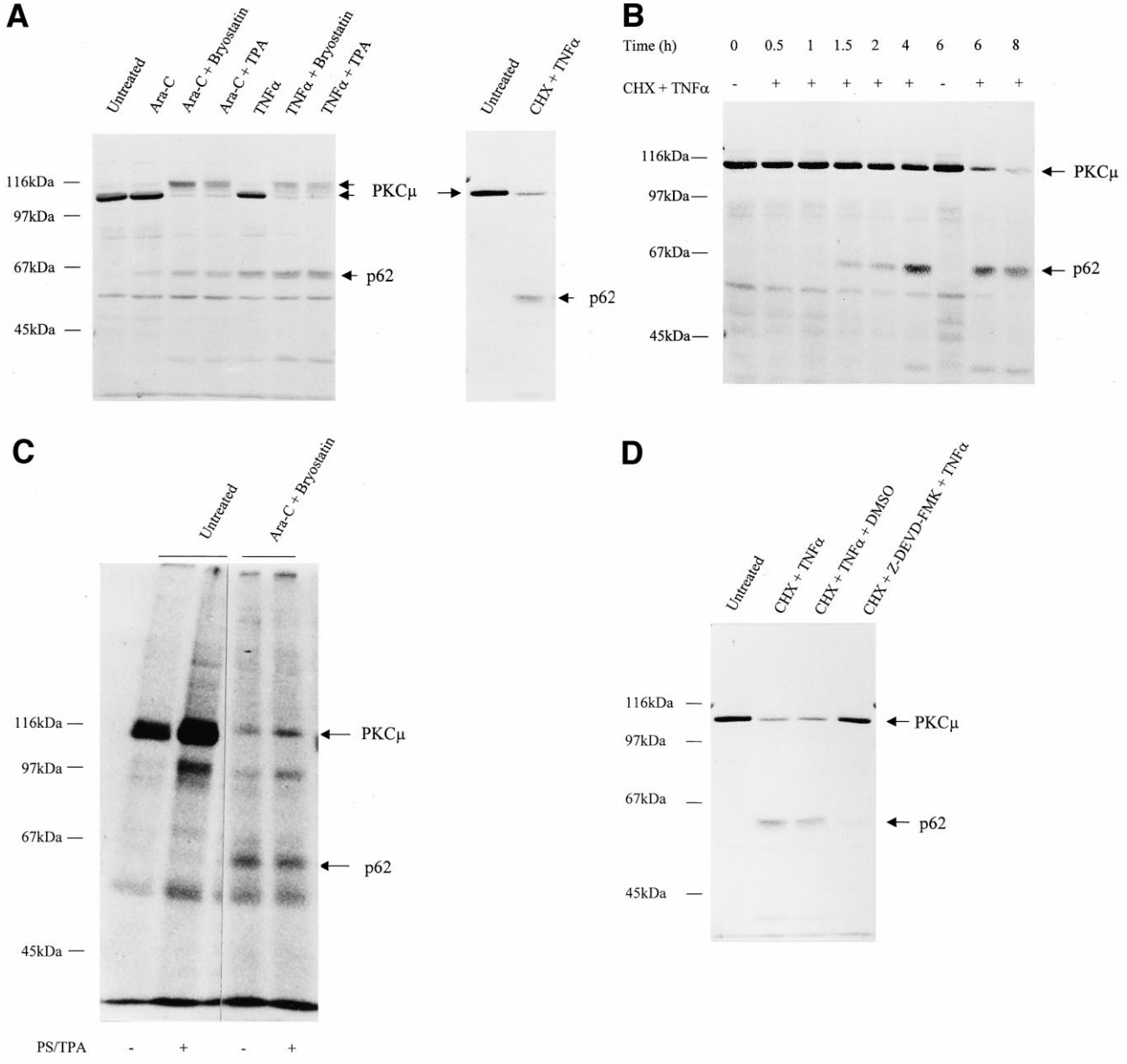

Fig. 1. Proteolytic cleavage of PKCμ in U937 cells upon treatment with various apoptosis-inducing agents. A: U937 cells remained untreated or were treated for 6 h with 10 μM ara-C alone or together with 300 nM TPA or 300 nM bryostatin or with 50 ng/ml TNFα alone or together with TPA or bryostatin (300 nM each). In another experiment, U937 cells were pretreated with 5 μg/ml cycloheximide (CHX) for 30 min and then treated with 50 ng/ml TNFα for 6 h. Cells were washed twice with PBS, resuspended in sample buffer and analyzed by SDS-PAGE. PKCμ and its fragment p62 (see arrows) were detected by immunoblotting using the PKCμ specific antibody sc-639 raised against a C-terminal peptide of PKCμ. B: Cycloheximide (CHX) and TNFα were applied to the cells as above for various times. PKCμ and p62 were visualized by immunoblotting (see arrows). C: Cells remained untreated or were treated with ara-C plus bryostatin as in A. Cell extracts were immunoprecipitated and the precipitates phosphorylated in the absence or presence of PS/TPA and applied to SDS-PAGE as described in the Section 2. Phosphorylated PKCμ and p62 were visualized by autoradiography (see arrows). D: Upon pretreatment with 5 μg/ml cycloheximide (CHX, 30 min), cells were treated for 6 h either with 50 ng/ml TNFα alone, TNFα plus 40 μl of the solvent DMSO or TNFα plus 100 μM caspase-3 inhibitor Z-DEVD-FMK in 40 μl DMSO. Preparation and processing of cell extracts were as in A.

2.4. Cleavage of recombinant PKCμ with caspase-3

Ten microliters of recombinant PKCμ was incubated with and without 16 μg/ml recombinant caspase-3 at 37°C for 15 min and the reaction products were analyzed by immunoblotting or phosphorylated as described below under Section 2.8 and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7.5%) and autoradiography of the gels.

2.5. In vitro translation of PKCμ wild-type and mutants

The PKCμ mutants (D391/A), (D349/A), (D388/A, D391/A), (D386/A), (D384/A) and (D378/A) were generated in two stages using the 'overlap extension' method as described previously [21]. [^{35}S]Methionine-labelled proteins (PKCμ wild-type and mutants) were synthesized by coupled transcription and translation reaction using the 'TNT Coupled Reticulocyte Lysate System' (Promega). Labelled proteins were incubated with and without 16 μg/ml caspase-3 at 37°C for 15 min. Reaction products were analyzed by SDS-PAGE (7.5%) and autoradiography of the gels.

2.6. Bacterial expression of recombinant GST-p62

The p62 fragment of PKCμ (amino acid residues 379–912) was amplified from the full-length PKCμ cDNA, cloned into the bacterial expression plasmid pGEX-2T (Pharmacia), expressed as a GST fusion protein and purified by affinity chromatography on glutathione beads. This construct is termed GST-p62. Primers used to amplify this region were 5′-GGGGGATCCAGTGGCGAGATGCAAGATCCAGACCCA and 5′-GGGGAATTCAGAGGATGCTGACACGCTCACCGAGGGCTT. The GST fusion protein of the fragment 392–912 was prepared in a similar way. GST was removed by treating the fusion proteins with 5 U/ml thrombin at room temperature for 2 h.

2.7. Protein kinase assay

Phosphorylation reactions with syntide 2 as substrate were carried out as described previously [12].

2.8. Autophosphorylation and phosphorylation of aldolase

Phosphorylation reactions were performed essentially as described for the protein kinase assay [12], but 37 μM ATP containing 8 instead of 1 μCi [γ-^{32}P]ATP was added. Moreover, the substrate was omitted (autophosphorylation) or aldolase (5 μg) was added instead of syntide 2 as a substrate. Proteins of the reaction mixture were separated by SDS-PAGE and visualized by autoradiography.

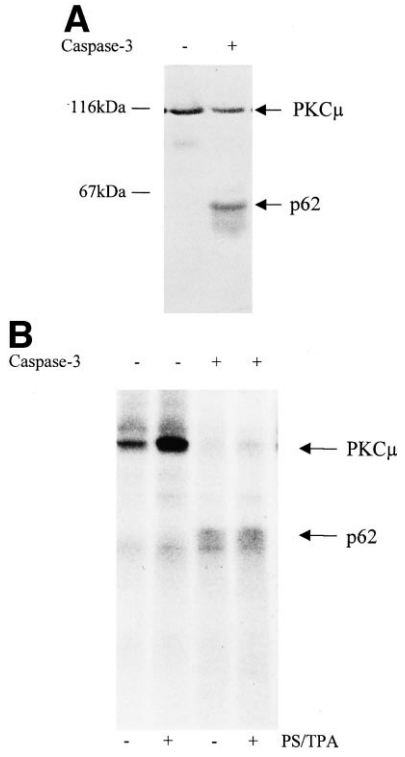

Fig. 2. Proteolytic cleavage of recombinant PKCμ by treatment with caspase-3 in a cell-free system. A: Ten microliters of recombinant PKCμ (a c-myc epitope-tagged PKCμ expressed in baculovirus-infected insect cells, see [36]) was incubated with or without 16 μg/ml caspase-3 at 37°C for 15 min. Upon SDS-PAGE, PKCμ and its fragment p62 were visualized by immunoblotting (see arrows). B: Recombinant PKCμ was treated as in A. Subsequently, the mixture was phosphorylated in the absence or presence of 10 μg PS and 100 nM TPA as described in Section 2. Phosphorylated PKCμ and p62 were visualized by autoradiography (see arrows).

3. Results and discussion

3.1. Generation of a 62 kDa fragment of PKCμ in vivo and in vitro

We noticed that between the regulatory and catalytic domain, human PKCμ contains two cleavage motifs of the classical type DXXD for the cysteinyl aspartate specific protease caspase-3. Therefore, we became interested in the question whether induction of apoptosis and, in this context, activation of caspase-3 might result in a specific fragmentation of PKCμ.

U937 cells were treated with ara-C or TNFα, i.e. agents known to induce apoptosis in these cells [37–39]. These treatments resulted in the generation of a PKCμ fragment with an apparent molecular weight of 62 kDa (p62), as demonstrated by immunoblotting using an antibody directed against a C-terminal peptide of PKCμ (Fig. 1A). The addition of bryostatin or TPA as well as the pretreatment for 30 min with cycloheximide augmented the effect of both agents on the fragmentation of PKCμ (Fig. 1A). Bryostatin and TPA alone were inactive in this respect (not shown). However, as previously described [13], these compounds caused a mobility shift of PKCμ, indicating its activation and (auto)phosphorylation. The combined effect of cycloheximide and TNFα was observed as early as 1.5 h upon treatment and reached a maximum at around 6 h (Fig. 1B). The fragment p62 was still able to autophosphorylate (Fig. 1C). In contrast to the intact PKCμ, however, its autophosphorylation could not be increased by PS/TPA.

The peptide Z-DEVD-FMK, an inhibitor of caspase-3, suppressed the TNFα/cycloheximide-induced cleavage of PKCμ (Fig. 1D), indicating that caspase-3 is the responsible enzyme. This conclusion was supported by the exclusive formation of a 62 kDa fragment upon treatment of recombinant PKCμ from baculovirus-infected insect cells in a cell-free system with caspase-3 for 15 min (Fig. 2A). Like the p62 fragment found in cells, the fragment produced by caspase-3 in vitro showed autophosphorylation that did not respond to PS/TPA (Fig. 2B). Another fragment of about 35 kDa was found only in vivo (Fig. 1A,B) but not in the cell-free system (Fig. 2A), indicating that it was not due to cleavage by caspase-3.

3.2. Identification of the cleavage site of PKCμ for caspase-3

To identify the caspase-3 cleavage site, we produced various aspartic acid to alanine mutants of PKCμ using an in vitro transcription/translation system and treated the cell extracts thus obtained with caspase-3. The proteolytic cleavage was determined by autoradiography of the ^{35}S-labelled proteins upon SDS-PAGE (Fig. 3). PKCμ contains a typical caspase-3 cleavage motif, i.e. $D^{388}HED^{391}$, which upon cleavage at D^{391}/A^{392} would give rise to a 62 kDa fragment. However, the D391A and D391A/D388A mutants were cleaved by caspase-3 as effectively as the wild-type (Fig. 3). Mutation of

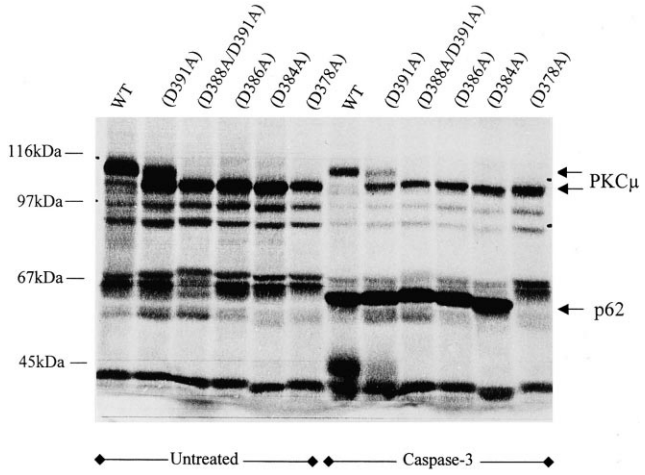

Fig. 3. Proteolytic cleavage of in vitro transcribed/translated PKCμ wild-type (WT) and mutants by caspase-3. PKCμ wild-type and mutants were synthesized in an in vitro transcription/translation system as described in Section 2. Various aspartic acid residues (D) were mutated to alanine (A) as indicated. The ^{35}S-labelled proteins were incubated with or without 16 μg caspase-3 at 37°C for 15 min. Upon SDS-PAGE, labelled proteins were visualized by autoradiography. PKCμ and p62 are indicated by arrows. In some experiments, a slower migrating form of PKCμ (possibly phosphorylated) is visible that has been observed previously [13].

another potential cleavage site for caspase-3, i.e. D^{349}/S^{350}, was equally ineffective (not shown). Therefore, we mutated other aspartic acid residues in this region, i.e. D^{378}, D^{384} and D^{386}, even though these are atypical cleavage sites for caspase-3. Whereas mutation of D^{384} and D^{386} to alanine did not affect cleavage, the D^{378}/A mutant was completely resistant to caspase-3 (Fig. 3). This demonstrated that PKCμ is cleaved by caspase-3 at the atypical site CQND^{378}/S^{379}. Atypical cleavage sites for caspase-3 have been reported previously [40–42].

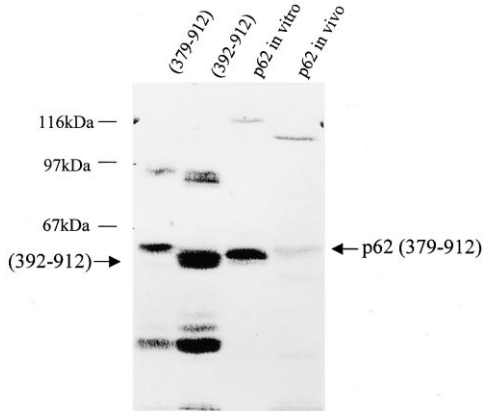

Fig. 4. Comparison of recombinant fragments of PKCμ with the p62 fragments produced in vitro and in vivo. Upon removal of the GST-tag by thrombin treatment (see Section 2), the bacterially expressed recombinant fragments (379–912) and (392–912) of PKCμ were applied to SDS-PAGE. In addition, caspase-3-treated PKCμ (p62 in vitro, see Fig. 2A) and an extract from cells treated with cycloheximide plus TNFα (p62 in vivo; see Fig. 1A,B) were applied to the gel. The recombinant fragments and p62 were visualized by immunoblotting.

3.3. Expression and characterization of the GST-tagged fragment 379–912 (GST-p62)

Two GST-tagged fragments were expressed in bacteria and partially purified by affinity chromatography, i.e. the fragment 379–912 corresponding to the atypical site D^{378}/S^{379} and the fragment 392–912 corresponding to the typical caspase-3 site D^{391}/A^{392}. Upon removal of the GST-tag with thrombin, the recombinant fragments were compared with the in vivo and in vitro fragments of PKCμ by immunoblotting. Fig. 4 shows that the latter fragments are identical in size with the recombinant fragment 379–912 but not with the fragment 392–912, indicating that caspase-3 indeed cleaves PKCμ at the atypical site D^{378}/S^{379} in vitro and in vivo.

The GST-tagged fragment 379–912 (GST-p62) was further characterized. Compared to intact PKCμ, GST-p62 exhibited

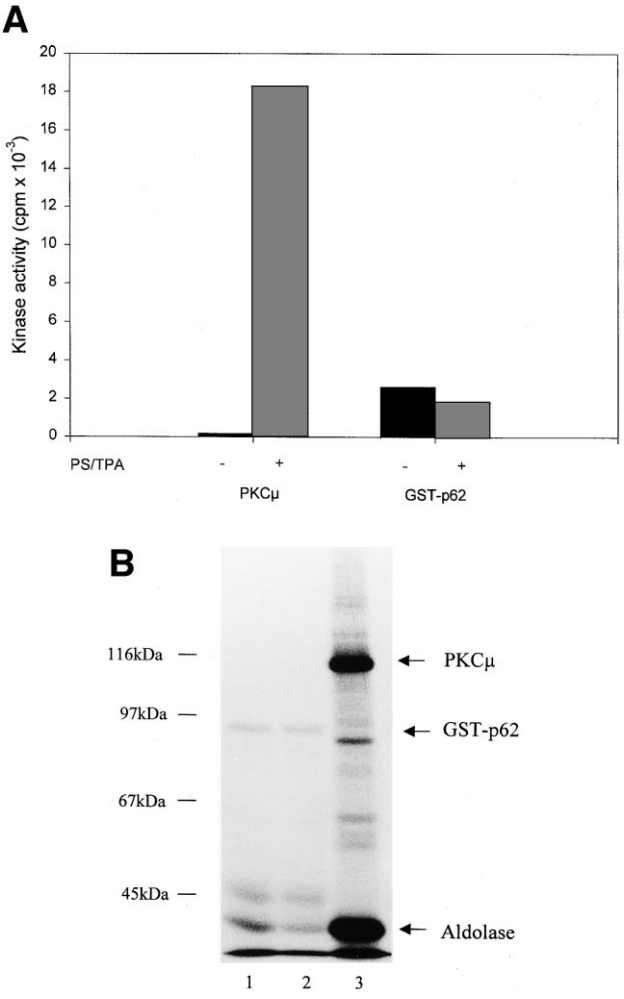

Fig. 5. Kinase activity of the recombinant fragment GST-p62. A: Equal amounts (as estimated by immunoblotting) of PKCμ (5 μl) or GST-p62 (2 μg protein) were used to phosphorylate syntide 2 and incorporation of phosphate was determined as described in Section 2. Phosphorylation of 5 μg syntide 2 was performed in the absence or presence of 10 μg PS/100 nM TPA. B: Five micrograms of aldolase was phosphorylated with equal amounts of PKCμ (3) or GST-p62 (2) in the presence of PS/TPA as described in Section 2. Phosphorylation with GST-p62 is shown also in the absence of PS/TPA (1) to allow for a comparison of the autophosphorylation of the recombinant fragment in the absence and presence of PS/TPA. Upon SDS-PAGE, phosphorylated proteins were visualized by autoradiography.

an around 15 times higher kinase activity in the absence of PS and TPA. In contrast to PKCμ, however, this basal kinase activity of the fragment could not be increased by PS/TPA, supporting the data on autophosphorylation of p62 in vivo (Fig. 1C) and in vitro (Fig. 2B). In fact, in the presence of PS/TPA, the fragment was much less active than intact PKCμ, as shown by phosphorylation of syntide 2 and aldolase (Fig. 5). Removal of the GST-tag did not affect the kinase activity of the fragment (not shown). Moreover, the fragment p62 was found to be more sensitive than PKCμ towards the kinase inhibitors K252a (IC_{50} of 0.6 nM for the fragment as compared to 7 nM for the intact PKCμ) and Gö6983 (IC_{50} of 2 μM versus 20 μM for intact PKCμ).

Considering these results, it is conceivable that the PKCμ fragment exhibits specific functions differing from those of the intact enzyme. In this context, it should be noted that localization experiments using GFP constructs indicate a differential subcellular localization of PKCμ and its fragment (Häussermann et al., unpublished observation). It has been reported that PKCμ might protect cells against apoptosis [43]. Thus, proteolytic cleavage of PKCμ upon induction of apoptosis possibly would abolish the protective effect. Moreover, the generated fragment might somehow support the apoptotic process (compare e.g. PKCδ [34]).

Acknowledgements: This work was supported by the Wilhelm Sander-Stiftung (97.090.1).

References

[1] Mellor, H. and Parker, P.J. (1998) Biochem. J. 332, 281–292.
[2] Parker, P.J. and Dekker, L.V. (1997) Protein Kinase C, Springer, London.
[3] Marks, F. and Gschwendt, M. (1996) in: Protein Phosphorylation (Marks, F., Ed.), pp. 81–116, VCH, Weinheim.
[4] Blobe, G.C., Stribling, S., Obeid, L.M. and Hannun, Y.A. (1996) Cancer Surv. 27, 213–248.
[5] Gschwendt, M. (1999) Eur. J. Biochem. 259, 555–564.
[6] Johannes, F.-J., Prestle, J., Eis, S., Oberhagemann, P. and Pfizenmaier, K. (1994) J. Biol. Chem. 269, 6140–6148.
[7] Valverde, A.M., Sinnett-Smith, J., Van Lint, J. and Rozengurt, E. (1994) Proc. Natl. Acad. Sci. USA 91, 8572–8576.
[8] Van Lint, J., Sinnett-Smith, J. and Rozengurt, E. (1995) J. Biol. Chem. 270, 1455–1461.
[9] Johannes, F.-J., Prestle, J., Dieterich, S., Oberhagemann, P., Link, G. and Pfizenmaier, K. (1995) Eur. J. Biochem. 227, 303–307.
[10] Dieterich, S., Herget, T., Link, G., Böttinger, H., Pfizenmaier, K. and Johannes, F.-J. (1996) FEBS Lett. 381, 183–187.
[11] Gibson, T.J., Hyvönen, M., Musacchio, A. and Saraste, M. (1994) Trends Biochem. Sci. 19, 343–347.
[12] Gschwendt, M., Dieterich, S., Rennecke, J., Kittstein, W., Müller, H.-J. and Johannes, F.-J. (1996) FEBS Lett. 392, 77–80.
[13] Rennecke, J., Johannes, F.-J., Richter, K.H., Kittstein, W., Marks, F. and Gschwendt, M. (1996) Eur. J. Biochem. 242, 428–432.
[14] Iglesias, T. and Rozengurt, E. (1999) FEBS Lett. 454, 53–56.
[15] Iglesias, T. and Rozengurt, E. (1998) J. Biol. Chem. 273, 410–416.
[16] Gschwendt, M., Johannes, F.-J., Kittstein, W. and Marks, F. (1997) J. Biol. Chem. 272, 20742–20746.
[17] Gschwendt, M., Kittstein, W. and Johannes, F.-J. (1998) FEBS Lett. 421, 165–168.
[18] Cazaubon, S., Bornancin, F. and Parker, P.J. (1994) Biochem. J. 301, 443–448.
[19] Orr, J.W. and Newton, A.C. (1994) J. Biol. Chem. 269, 27715–27718.
[20] Keranen, L.M., Dutil, E.M. and Newton, A.C. (1995) Curr. Biol. 5, 1394–1403.
[21] Stempka, L., Girod, A., Müller, H.-J., Rincke, G., Marks, F., Gschwendt, M. and Bossemeyer, D. (1997) J. Biol. Chem. 272, 6805–6811.
[22] Stempka, L., Schnölzer, M., Radke, S., Rincke, G., Marks, F. and Gschwendt, M. (1999) J. Biol. Chem. 274, 8886–8892.
[23] Zugaza, J.L., Sinnett-Smith, J., Van Lint, J. and Rozengurt, E. (1996) EMBO J. 15, 6220–6230.
[24] Ashkenazi, A. and Dixit, V.M. (1998) Science 281, 1305–1308.
[25] Salvesen, G.S. and Dixit, V.M. (1997) Cell 91, 443–446.
[26] Thornberry, N.A. and Lazebnik, Y. (1998) Science 281, 1312–1316.
[27] Cardone, M.H., Salvesen, G.S., Widmann, C., Johnson, G. and Frisch, S.M. (1997) Cell 90, 315–323.
[28] Deak, J.C., Cross, J.V., Lewis, M., Qian, Y., Parrot, L.A., Distelhorst, C.W. and Templeton, D.J. (1998) Proc. Natl. Acad. Sci. USA 95, 5595–5600.
[29] Widmann, C., Gerwins, P., Johnson, N.I., Jarpe, M.B. and Johnson, G.L. (1998) Mol. Cell. Biol. 18, 2416–2429.
[30] Takahashi, M., Mukai, H., Toshimori, M., Miyamoto, M. and Ono, Y. (1998) Proc. Natl. Acad. Sci. USA 95, 11566–11571.
[31] Emoto, Y., Manome, Y., Meinhardt, G., Kisaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W.W., Kamen, R., Weichselbaum, R. and Kufe, D. (1995) EMBO J. 14, 6148–6156.
[32] Ghayur, T., Hugunin, M., Talanian, R.V., Ratnofsky, S., Quinlan, C., Emoto, Y., Pandey, P., Datta, R., Kharbanda, S., Allen, H., Kamen, R., Wong, W. and Kufe, D. (1996) J. Exp. Med. 184, 2399–2404.
[33] Mizuno, K., Noda, K., Apaki, T., Imaoka, T., Kobayashi, Y., Akita, Y., Shimonaka, M., Kishi, S. and Ohno, S. (1997) Eur. J. Biochem. 250, 7–18.
[34] Widmann, C., Gibson, S. and Johnson, G.L. (1998) J. Biol. Chem. 273, 7141–7147.
[35] Frutos, S., Moscat, J. and Diaz-Meco, M.T. (1999) J. Biol. Chem. 274, 10765–10770.
[36] Dieterich, S., Herget, T., Link, G., Böttinger, H., Pfizenmaier, K. and Johannes, F.-J. (1996) FEBS Lett. 381, 183–187.
[37] Vanags, D.M., Pörn-Ares, M.I., Coppola, S., Burgess, D.H. and Orrenius, S. (1996) J. Biol. Chem. 271, 31075–31085.
[38] Kaufmann, S.H. (1989) Cancer Res. 49, 5870–5878.
[39] Gunji, H., Kharbanda, S. and Kufe, D. (1991) Cancer Res. 51, 741–743.
[40] Satoh, S., Hijikata, M., Handa, H. and Shimotohno, K. (1999) Biochem. J. 342, 65–70.
[41] Park, J.A., Kim, S.I. and Lee, S.K. (1998) Eur. J. Biochem. 257, 242–248.
[42] Caulin, C., Salvesen, G.S. and Oshima, R.G. (1997) J. Cell Biol. 138, 1379–1394.
[43] Johannes, F.J., Horn, J., Link, G., Haas, E., Siemienski, K., Wajant, H. and Pfizenmaier, K. (1998) Eur. J. Biochem. 257, 47–54.