Stress-induced Activation of Protein Kinase CK2 by Direct Interaction with p38 Mitogen-activated Protein Kinase*

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Protein kinase CK2 has been implicated in the regulation of a wide range of proteins that are important in cell proliferation and differentiation. Here we demonstrate that the stress signaling agents anisomycin, arsenite, and tumor necrosis factor-α stimulate the specific enzyme activity of CK2 in the human cervical carcinoma HeLa cells by up to 8-fold, and this could be blocked by the p38 MAP kinase inhibitor SB203580. We show that p38α MAP kinase, in a phosphorylation-dependent manner, can directly interact with the α and β subunits of CK2 to activate the holoenzyme through what appears to be an allosteric mechanism. Furthermore, we demonstrate that anisomycin- and tumor necrosis factor-α-induced phosphorylation of p38 at Ser-392, which is important for the transcriptional activity of this growth suppressor protein, requires p38 MAP kinase and CK2 activities.

The signaling pathways that regulated protein kinase CK21 (formerly casein kinase II) have been elusive since the discovery of this highly conserved and ubiquitous protein-serine/threonine kinase over 45 years ago (1–5). Despite the identification of over 160 putative substrates for CK2, the overriding function of this kinase has been evasive. In most cells, the holoenzyme form of CK2 is a constitutively active heterotetramer of two catalytic α and/or α′ and two regulatory (β) subunits, but CK2β-free pools of CK2α also exist. Further increases (2–3-fold) in the phosphotransferase activity of CK2 in cell lysates toward synthetic peptide substrates have been reported following treatment of murine and human fibroblastic cell lines, A431 and 3T3-L1 adipocytes with serum (6), insulin (7–9), insulin-like growth factor-1 (10), epidermal growth factor (7, 11), bombesin (12), and tumor necrosis factor (TNF) (13). CK2 phosphotransferase activity and protein levels are also commonly elevated in solid human tumors and transformed cell lines (2–4, 14, 15). Furthermore, CK2α overexpression along with c-Myc induces lymphomas in mice (16). While the increased expression of CK2 is linked with neoplastic transformation, little is known concerning acute regulation of this protein kinase.

EXPERIMENTAL PROCEDURES

Cell Culture and Lysates Preparation—HeLa cells were grown at 37°C on 125-cm2 flasks in M199 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum. For cell stimulation, HeLa cells were split at 2 × 106 cells/10-cm2 dish 24 h prior to starvation. Cells were starved with 0.5% fetal calf serum for 15–20 h before exposure to stimuli. Starved cells were incubated with 10 μM SB203580 (Calbiochem) for 30 min prior to addition of 10 μM anisomycin (Sigma) for 30 min, 50 μM arsenite (Sigma) for 15 min, or 20 ng/ml TNFα for 15 min. Adhering cells were washed twice with ice-cold phosphate-buffered saline, scraped, then lysed in 50 mM Hepes (pH 7.2), 100 mM NaCl, 1 mM EDTA, and 20 mM sodium chloride in Buffer A (1 mM sodium orthovanadate, 1% aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 0.5 μM leupeptin, 10 μg/ml soybean trypsin inhibitor, and 1% Nonidet P-40). Lysates were subjected to brief sonication for 2 × 10 s, centrifuged at 15,000 rpm for 5 min, and the supernatants were collected and stored frozen prior to use. Protein was determined by the method of Bradford (17) using bovine serum albumin as a standard.

In some experiments, anisomycin-treated cells were lysed with ice-cold 100 mM Tris-HCl (pH 8), 100 mM NaCl, 50 mM MgCl2, 0.3 mM EDTA, 0.5 mM ZnCl2, and 1% Nonidet P-40. After sonication for 2 × 10 s, the lysates were centrifuged at 14,000 rpm for 5 min and the supernatant was incubated with 0.5 units/μg alkaline phosphatase (Calbiochem) for 2 h at 37°C. Ten volumes of ice-cold Buffer B (10 mM MOPS (pH 7.2), 25 mM β-glycerophosphate, 2 mM EDTA, 5 mM EGTA, and 2 mM sodium orthovanadate) were added and the sample was subjected to MonoQ anion-exchange chromatography.

MonoQ Chromatography and Immunoprecipitation—Cell lysates (2 mg protein) were diluted 1:10 in Buffer B and loaded onto a MonoQ (1 ml; Amersham Pharmacia Biotech) column at 4°C. The anion-exchange column was eluted with a 20-ml linear gradient of 0–0.8 M NaCl in Buffer B and 0.5–ml fractions were collected.

Immunoprecipitations were performed with a rabbit polyclonal antibody for p88ε (Santa Cruz Biotechnology), and a mouse monoclonal antibody for CK2δ. Cell extracts (0.5 mg) were preincubated for 1 h with 20 μl of protein A-Sepharose, and then for 30 min with 20 μl of protein-A Sepharose that had been blocked with 1% bovine serum albumin. The preincubated lysates were further incubated with 4 μg of the appropriate antibody and 20 μl of protein A-Sepharose in the presence of 0.1% SDS for 3 h at 4°C. The beads were washed four times with 20 mM Tris-HCl (pH 7.4) and 150 mM NaCl in Buffer A.

CK2 Activity Assays and Immunoblots—CK2 activity following column chromatography was assayed when 5 μl of MonoQ fractions were incubated in a final volume of 25 μl with 5 μg of partially phosphorylated casein and 100 μM [γ-32P]ATP (2.5 μCi/assay) in Buffer C (12 mM MOPS (pH 7.2), and 15 mM MgCl2) for 10 or 15 min at 30°C. These and other CK2 assays were performed in the absence and presence of either 20 μg/ml heparin (Sigma), 20 μM DRB (Sigma) or 20 μM SB203580 (Calbiochem). In cytosolic extracts, CK2 activity was assayed with 0.5 mM substrate peptide RRADDSDDDDDD (Calbiochem). The phosphorylation of casein was quantitated by spotting 20 μl on to...
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RESULTS AND DISCUSSION

To explore the role of stress stimuli in the regulation of CK2, we investigated the effect of a 30-min treatment of serum-deprived HeLa cells with the protein synthesis inhibitor anisomycin. The casein phosphotransferase activity of CK2 was assessed following MonoQ fast protein liquid chromatography of the cell lysates. Anisomycin treatment caused an 8-fold activation of CK2, which eluted with 6.6 mg NaCl in primarily fraction numbers 18 and 19 (Fig. 1A). This was confirmed by the sensitivity of the casein phosphotransferase activity peak to the CK2 inhibitor heparin (Fig. 1A), and the elution of the α, α’, and β subunits of CK2 in these MonoQ fractions as revealed by immunoblotting analysis (Fig. 2, A and D). Exposure of the serum-starved cells to the mitochondrial oxidant arsenite or the inflammatory cytokine TNF-α for 15 min was also observed to stimulate CK2 activity by 3–4-fold (Fig. 1B). Furthermore, using a highly specific CK2 substrate peptide (RRADDSDDDDD), cytosolic extracts from HeLa cells treated with arsenite or anisomycin, respectively, contained 0.5 ± 0.06 and 7.44 ± 0.03 (mean ± S.E., n = 3) fold more phosphotransferase activity than was detectable with cytosol from nontreated cells (data not shown).

Under the same conditions that activated CK2, anisomycin, arsenite, and TNF-α also produced marked increases in the phosphorylation of the p38 MAP kinase (also known as Hog, CSBP, MPK2, and SAPK2) as determined by immunoblotting with an antibody specific for the activating phosphorylation site of p38 isoforms (Fig. 1C) (20). To assess whether a p38 MAP kinase mediated stress factor-induced activation of CK2, we took advantage of the specific p38 inhibitor SB203580 (21–24). Treatment of the serum-deprived HeLa cells with SB203580 for 30 min prior to their exposure to anisomycin, arsenite, or TNF-α abrogated the ability of each of these agents to stimulate CK2 (Fig. 1, A and B).

As the anisomycin treatment consistently elicited the most striking activation of CK2, we exploited this agent to further explore the mechanism for the CK2 stimulation. There were no differences in the intensity of immunostaining of the α, α2γ, and β subunits of CK2 in serum-deprived anisomycin and SB203580-treated cells (Fig. 2, A–F). Consequently, the anisomycin appeared to increase the specific enzyme activity of CK2 without alteration of the α, α2γ/β subunit ratio or the total amount of CK2 protein. There were also no apparent reductions in the mobilities of the CK2 subunits following SDS-polyacrylamide gel electrophoresis of the MonoQ-fractionated lysates from cells exposed to anisomycin. Such retardation of CK2 subunits usually reflects their increased phosphorylation.

CK2 subunits have been reported to interact with many other proteins (4), including the protein kinases Mos, RafA, and Rsk, which bind to CK2β (25–28). Therefore, we examined whether a p38 MAP kinase might directly associate with CK2. The peak of immunoreactivity of the α-isoform of p38 was found to coelute with CK2 following MonoQ fractionation, and this was most pronounced in lysates from anisomycin-treated cells (Fig. 2, G and H). By contrast, the amount of p38α coeluting with CK2 was substantially reduced in extracts from cells treated with both anisomycin and SB203580, and a large portion of the MAP kinase failed to bind to the MonoQ column and eluted in wash-through fractions 4–7 (Fig. 2J). An even more dramatic redistribution of p38α away from CK2 was produced when the lysates from cells exposed to anisomycin were incu-
bated with alkaline phosphatase to dephosphorylate the p38α prior to MonoQ chromatography (Fig. 2J). CK2 subunits were not detectable by immunoblotting in MonoQ fractions 4–7 neither before nor after alkaline phosphatase treatment. Furthermore, the alkaline phosphatase treatment abolished the anisomycin-induced activation of CK2 (data not shown). An implication of these experiments was that p38α MAP kinase must be in an active conformation in order to maximally bind and activate CK2.

While the preceding data for CK2 and p38α residing in a common complex was only correlative, direct evidence was obtained through immunoprecipitation studies. The p38α antibody was used to immunoprecipitate equivalent amounts of p38α from lysates of serum-deprived, anisomycin-, and SB203580 + anisomycin-treated cells (Fig. 3B). The casein phosphotransferase activity was approximately 4-fold higher in the immunocomplexes from anisomycin-treated as compared with untreated cells, and this was reduced to less than a 2-fold stimulation by the SB203580 (Fig. 3A). These immunocomplexes with p38α antibody were shown to contain both the α and β subunits of CK2 by immunoblotting; and their presence was increased in the immunocomplexes from anisomycin-treated cells (Fig. 3, C and D). Reciprocally, in immunocomplexes obtained with an antibody specific for CK2β, p38α was largely immunodetected in samples from anisomycin-treated cells, with little if any evident in immunocomplexes from serum-starved or SB203580 + anisomycin-treated cells (Fig. 3E).

Further support for the direct interaction of CK2 and p38α was obtained through the use of recombinant CK2 and partially active p38α expressed as a GST fusion protein. GST-p38 displayed binding for both α and β subunits of CK2 when they were expressed separately (data not shown). Therefore p38α can interact with both CK2 subunits independently. The interaction of CK2 for GST-p38α was specific, since no binding of CK2α was detected when either GST or GST fused to the MAP kinase Erk1 was substituted for GST-p38α (Fig. 4C). GST-p38α stimulated the casein phosphotransferase activity of recombinant CK2 by 2-fold, and this was sensitive to SB203580 and the CK2 inhibitors heparin and 5,6-dichloro-1-benzimidazole (DRB) (Fig. 4A). When the CK2/GST-p38α complex immobilized on glutathione-Sepharose was incubated with [γ-32P]ATP, there was strong phosphorylation of the β-subunit of CK2 (Fig. 4B), but not the α-subunit of CK2 (data not shown). This phosphorylation was abolished when the incubations with [γ-32P]ATP were carried out with SB203580, DRB, or heparin. When the presence of CK2α was ascertained by immunoblotting, the DRB and heparin did not dissociate the CK2/GST-p38α complex, whereas SB203580 did (Fig. 4C). These findings indicated that the radiolabeling of CK2β in the presence of GST-p38α was largely due to autophosphorylation, apparently from the stimulation of CK2 catalytic activity. This was further supported by experiments in which the CK2 was preincubated with unlabeled ATP for 15 min to saturate the autophosphorylation sites, and then with [γ-32P]ATP in the absence and presence of GST-p38α for an additional 10 min. Under these circumstances, there was no enhanced incorporation of radioactivity into the α and β subunits of CK2 with the addition of GST-p38α (data not shown).

Collectively, our results support a model in which phosphorylated p38α is able to engage CK2 in a complex through direct interaction with both the α and β subunits of CK2. It would
appear that an active conformation of p38α is needed for binding, since the SB203580 prevented complex formation. Through an allosteric effect exerted by active p38α, CK2 may become stimulated to phosphorylate other targets. It is unclear if the increased autophosphorylation of CK2 may be sufficient to preserve the state of activation of CK2 after p38α is dissociated. The ability of 4-fold lower levels of p38α to activate CK2 in vitro is consistent with this possibility.

The functional consequences of the stress activating of CK2 may include activation of the p53 growth suppressor protein. Phosphorylation at Ser-392 in human p53 (equivalent to Ser-389 in murine p53) is known to increase its DNA binding ability and transcriptional activity (29–31). Recently, Huang et al. (33) have found that CK2 is also co-immunoprecipitated with p53. Our experiments support a model in which a p38-CK2 complex binds to p53, and the direct phosphorylation of Ser-389 in response to UV radiation is catalyzed by CK2.

To test the hypothesis that the p53 is a downstream target of the stress stimuli anisomycin, arsenite, and TNFα, we performed immunoblotting studies of lysates from HeLa cells that had been treated with these factors using a p53 Ser-392 phosphorylation site-specific antibody. As shown in Fig. 5, the treatment of serum-deprived HeLa cells with any of these agents enhanced the phosphorylation of p53 at this site. The phosphorylation of p53 in response to anisomycin and TNFα, but not arsenite, was markedly attenuated by either DRB or SB203580. This indicated that the phosphorylation of p53 at this activating site in response to anisomycin or TNFα was mediated by p38 MAP kinase and CK2. In the case of arsenite, there may be an alternative pathway, in addition to the p38 MAP kinase-CK2 route, by which p53 can be phosphorylated at the Ser-392 site.

The implication of CK2 in mediation of stress signaling downstream of p38 MAP kinase is a surprising outcome of our study. Our findings underline the importance of phosphatase inhibitors to preserve the state of CK2 activation and the potential influence of agents that are commonly used to inhibit cellular processes (e.g. protein synthesis, mitochondrial function, and cell cycle progression) in order to study the regulation and role of CK2.

REFERENCES

1. Burnett, G., and Kennedy, E. P. (1954) J. Biol. Chem. 211, 969–980
2. Pina, L. A., and Meggio, F. (1997) Cell Cycle Res. 3, 77–96
3. Heriche, J. K., and Chambaz, E. M. (1998) Oncogene 17, 13–18
4. Guerra, B., and Issinger, O. G. (1999) Electrophoresis 20, 391–408
5. Dobrowolska, G., Lozeman, F. J., Li, D., and Krebs, E. G. (1999) Mol. Cell. Biochem. 191, 3–12
6. Carroll, D., and Marshall, D. R. (1989) J. Biol. Chem. 264, 7345–7348
7. Sommercorn, J., Mulligan, J. A., Lozeman, F. J., and Krebs, E. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8834–8838
8. Klarland, J. K., and Czech, M. P. (1988) J. Biol. Chem. 263, 15872–15875
9. Villa-Moruzzi, E., and Crabb, J. W. (1993) Biochem. Biophys. Res. Commun. 177, 1019–1024
10. Wang, L. G., Liu, X. M., Wikel, H., and Bloch, A. (1995) J. Leukocyte Biol. 57, 332–334
11. Ackerman, P., and Osheroff, N. (1989) J. Biol. Chem. 264, 11958–11965
12. Agostini, P., Van Lint, J., Sarno, S., De Witte, P., Vandenheede, J. R., and Merlevede, W. (1992) J. Biol. Chem. 267, 9732–9737
13. Van Lint, J., Agostinis, P., Vandevorde, V., Haegeman, G., Fiers, W., Merlevede, W., and Vandenheede, R. J. (1992) J. Biol. Chem. 267, 25916–25921
14. Prowse, K., Fischer, H., and Issinger, O.-G. (1984) FEBS Lett. 176, 479–483
15. Münstermann U., Friz, G., Seitz, G., Viping, L., Schneider, H. R., and Issinger, O.-G. (1990) Eur. J. Biochem. 198, 251–257
16. Seldin, D. C., and Leder, P. (1995) Science 267, 894–897
17. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
18. Laemmli, U. K. (1970) Nature 227, 680–684
19. Charlton, L., Sanghera, J. S., Clark-Lewis, I., and Pelech, S. L. (1992) J. Biol. Chem. 267, 8840–8845
20. Paul, A., Wilson, S., Belham, C. M., Robinson, C. J. M., Scott, M. H., Gould, G. W., and Plevin, R. (1997) Cell Signal. 9, 403–410
21. Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, R. J., Landvatter, S. W., Strickler, J. E., Melaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) Nature 372, 739–746
22. Cuenda, A., Rousse, J., Dox, Y. N., Meir, H., Cohen, P., Gallagher, T. F., Young, P. R., and Lee, J. C. (1995) FEBS Lett. 364, 229–233
23. Goedert, M., Cuenda, A., Craxton, M., Jakes, R., and Cohen, P. (1997) EMBO J. 16, 3563–3571
24. Takeda, K., Morishita, T., and Nishida, E. (1998) Science 280, 599–602
25. Bovetti, M. P., Massari, S., Finelli, P., Meggio, F., Boldyreff, B., Issinger, O.-G., Palumbo, G., Ciarcia, C., and Bonaccorsi, S. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6067–6071

FIG. 5. Stress factor-induced phosphorylation of p53 at Ser-392 is dependent on CK2 and p38 MAP kinase. Treatment of serum-deprived HeLa cells with anisomycin (10 μg/ml for 30 min), arsenite (50 μM for 15 min), or TNFα (20 ng/ml for 15 min) resulted in the enhanced phosphorylation of p53 as detected with a p53 Ser-392-phospho-site antibody (New England Biolabs). The increased phosphorylation in response to anisomycin and TNFα could be reduced by pretreatment of the cells with the CK2 inhibitor DRB (20 μM) and by the p38 MAP kinase inhibitor SB203580 (10 μM). Results are representative of three experiments.
26. Hagemann, C., Kalmes, A., Wixler, V., Wixler, L., Schuster, T., and Rapp, U. R. (1997) FEBS Lett. 403, 200–202
27. Boldyreff, B., and Issinger, O.-G. (1997) FEBS Lett. 403, 197–199
28. Kusk, M., Ahmed, R., Thomsen, B., Bendixen, C., Issinger, O.-G., and Boldyreff, B. (1999) Mol. Cell. Biochem. 191, 51–58
29. Hupp, T. R., and Lane, D. P. (1994) Curr. Biol. 4, 865–875
30. Sakaguchi, K., Sakamoto, H., Lewis, M. S., Anderson, C. W., Erickson, J. W., Appella, E., and Xie, D. (1997) Biochemistry 36, 10117–10124
31. Kapor, M., and Luzano, G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2834–2837
32. Huang, C., Ma, W.-Y., Maxiner, A., Sun, Y., and Dong, Z. (1999) J. Biol. Chem. 274, 12229–12235
33. Kraiss, S., Barnekow, A., and Montenarh, M. (1990) Oncogene 5, 845–855