Osmotic Stress Protein 94 (Osp94): A New Member of the Hsp110/SSE Gene Subfamily

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Preservation of cell viability and function in the hyperosmolar environment of the renal medulla is a complex process that requires selective gene expression. We have identified a new member of the heat shock protein (hsp) 70 superfamily that is up-regulated in renal inner medullary collecting duct cells (mIMCD3 cells) during exposure to hyperosmotic NaCl stress. Known as osmotic stress protein 94, or Osp94, this 2935-base pair cDNA encodes an 838-amino acid protein that shows greatest homology to the recently discovered hsp110/SSE gene subfamily. Like the hsps, Osp94 has a putative amino-terminal ATP-binding domain and a putative carboxy-terminal peptide-binding domain. The in vitro translated Osp94 product migrated as a 105–110-kDa protein on SDS-polyacrylamide gel electrophoresis. In mIMCD3 cells, Osp94 mRNA expression was greatly induced during water restriction when osmolarity is known to increase. Thus, Osp94 is a new member of the hsp110/SSE stress protein subfamily and likely acts as a molecular chaperone.

EXPERIMENTAL PROCEDURES

Cell culture and isolation of RNA—mIMCD3 cells (20) were grown to confluence on plastic dishes in Dulbecco's modified Eagle's medium/F12 (1:1) supplemented with 10% fetal bovine serum. For hyperosmotic stress experiments, cells were starved of serum for 24 h and then either maintained at 37 °C or exposed to elevated (42 °C) temperature; no recovery period was allowed. At an appropriate time point cells were washed twice with phosphate-buffered saline and total RNA was isolated using the RNAzol method (Tel-Test, Inc). Poly(A) ϩ RNA was isolated by using Oligotex-dT (Qiagen).

virus; GAPDH, glyceraldehyde-3-phosphatase dehydrogenase.
Differential mRNA Expression Analysis—Analysis of differential mRNA expression was performed using an RT-PCR reaction with arbitrary primers as described previously (21) with some minor modifications. For the reverse transcriptase reaction, a 20-μl reaction mixture containing 2 μg of total RNA, 10 units of RNase inhibitor (Promega), 2 mM dithiothreitol, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 5 mM MgCl₂, 100 μg/ml oligo(dT) primer, and 200 units of MMLV reverse transcriptase (Life Technologies, Inc.) was incubated at 1 h at 37 °C, heated to 99 °C for 5 min, and then chilled on ice. To perform PCR, 1 μl of the cDNA reaction mixture was added to 1.25 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 μM of each primer, 5 μCi of [32P]dATP, and 0.3 unit of Taq polymerase. Using a thermal cycler, all PCR reactions were performed as follows: 95 °C for 1 min, then 40 cycles of 94 °C for 15 s, 55 °C for 45 s, and 72 °C for 30 s, and then a final extension period at 72 °C for 10 min. The primers included in the PCR reaction were 5'-GACGAGACGTCTC-3' and 5'-GGCGAGTGTGAT-3' (Oligos Etc, Portland, OR). The PCR products were separated by electrophoresis on a denaturing 6% polyacrylamide-urea gel prepared using Seagel (National Diagnostics). Samples were run for 2-3 h at 1500 V, transferred to filter paper, and autoradiographed.

Cloning the cDNA Fragment—The cDNA fragment was excised from the gel, eluted into 0.5 mM ammonium acetate, 1 mM EDTA (pH 8.0), precipitated, and washed. Using PCR the cDNA product was reamplified in the presence of 2 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 20 μM dNTPs, 1.25 μM primers, and 2 units of Taq polymerase. The thermal cycling protocol was identical to that used in the initial PCR process described above.

Reamplified cDNA fragments were separated on a 2% agarose gel, isolated, blunt-end ligated into plBluescript SK⁺, and cloned. Plasmid DNA was isolated and the cDNA insert was sequenced at the Howard Hughes Biopolymers Research Facility at Harvard Medical School.

Cloning the Full-length Osp94 cDNA—A cDNA library was prepared using poly(A)⁺ RNA isolated from mIMCD3 cells exposed to hyperosmotic NaCl using the Superscript plasmid system (Life Technologies, Inc.). The library, consisting of 60,000 independent colonies, was probed with a 32P-labeled oligonucleotide probe prepared from the Osp94 PCR product using a labeling kit (Pharmacia Biotech, Inc.). The probe was hybridized to the filters for 20 h at 37 °C. The hybridization solution consisted of the 32P-labeled DNA probe (10⁶ cpm/ml), 10% dextran sulfate, 4× SSC, 25 min, once at 60 °C (0.2× SSC, 25 min), and then exposed to film. Two positive cDNA clones were identified and plaque-purified by performing a second round of screening. The full-length Osp94 cDNA was sequenced in both directions using a primer-walking strategy and sequence analysis, and final alignments were performed with GeneWorks 2.1.1 software (Intelligenetics).

Northern Analysis—Total (20 μg) or poly(A)⁺ (2 μg) RNA was fractionated on a 2% agarose gel, isolated, blunted-end ligated into pBluescript SK⁺, and cloned. Plasmid DNA was isolated and the cDNA insert was sequenced at the Howard Hughes Biopolymers Research Facility at Harvard Medical School.

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Osp94 has two structurally distinct domains: an amino-terminal domain that is highly charged, particularly the carboxyl terminus. There is no evidence of Osp94 containing a signal peptide sequence. The ARK (amino acids 123–125) may be a potential peroxisomal targeting signal sequence, although this remains speculative. There is no evidence of a transmembrane domain in Osp94.

In vitro translation of Osp94 analyzed by SDS-PAGE. Lane 1 (cDNA) shows a negative control using water instead of the cDNA. Lanes 2 and 3 show 35S-labeled protein products when Osp94 cDNA was translated in the absence or presence of pancreatic microsomes (micro).

Smaller protein products. In vitro translation in the presence of canine pancreatic microsomes had no major effect on the size or abundance of the 105–110-kDa protein product, although there appeared to be small changes in the smaller, minor products. The significance of the minor products is unknown, although they may represent inefficient translation start sites or, less likely, cleavage sites. Overall, these data suggest that the major protein product of Osp94 has a molecular mass of 105–110 kDa on SDS-PAGE.

Based on the cDNA sequence the deduced Osp94 protein (Fig. 4) contains 838 amino acids. The deduced protein contains putative myristylation sites and many consensus phosphorylation sites for protein kinase C, casein kinase 2, and tyrosine kinase. Hydrophobicity analysis using either the Kyte-Doolittle or Eisenberg algorithm indicated there are no transmembrane domains suggesting it is localized intracellularly. Whereas the subcellular localization of Osp94 remains to be defined, analysis of Osp94 using PSORT (22) indicated it may possess signal sequences that target the protein to mitochondria and peroxisomes but not nucleus though the degree of certainty (0.3–0.47) appeared to be relatively low. ARSGGI (amino acids 18–23) may represent a potential mitochondrial matrix signal and ARK (amino acids 123–125) may be a potential peroxisomal signal sequence, although this remains speculative. There is no evidence of Osp94 containing a signal peptide sequence. The deduced protein contains 15 cysteines localized predominantly in the amino half of the protein. Moreover, the deduced protein is highly charged, particularly the carboxyl terminus.

Like many other members of the hsp family of proteins, Osp94 has two structurally distinct domains: an amino-terminal putative ATP-binding domain and a carboxyl-terminal putative peptide-binding domain. The overlined amino acids in Fig. 4 indicate the five putative motifs of the ATP-binding domain that form a three-dimensional pocket for ATP binding and a putative interdomain hinge region that couples the ATP-binding domain to the substrate binding domain. The ATP-binding domain of Osp94 is more closely related to that found in a large class of ATPase molecules including the hsp70s, actin, and hexokinase (23). The carboxyl-terminal half of Osp94 contains no distinct consensus structural motifs. However, sequence homology to known peptide binding chaperone molecules suggests it functions as a peptide/protein binding domain.

Homology of Osp94 to the hsp110 gene subfamily—Homology searches of the protein data bases using BLASTX (24) showed that Osp94 is most related to numerous heat shock proteins. Of particular note and as indicated in Figs. 4 and 5, Osp94 was most identical to the recently cloned hsp110 from CHO cells (13) and hsp70RY, an orphan gene expressed in B-cells (14). Sequence alignment of these three proteins (Fig. 4) shows that homology extends over the entire length of Osp94 particularly in the amino half of the molecule. Notably, within the C-terminal half of Osp94 there are two regions of significant sequence divergence (Val497–Gln594 and Lys707–Asp838). Independent homology searches using BLASTP failed to identify proteins with homology to these nonconserved regions of Osp94.

An extensive homology analysis showed Osp94 is most similar to hsp110 (65% identical) (13) and hsp70RY (62% identical) (14). Osp94 also has lower but significant homology (30%) to the smaller hsp70 class of chaperones including the inducible (hsp70) and constitutive forms (hsc70) as well as the endoplasmic reticulum chaperone known as BiP or grp78 (25). Consistent with this observation, analysis of PROSITE protein sequence motifs showed Osp94 contains two of the three hsp70 protein family signatures. In particular signature 2 (FIDMGH-SAYQSVVC) and signature 3 (IEIVGGATRIPAVKE) were present in the amino half of the deduced protein. As for the hsp70 signature 1 motif, Osp94 contained the first 4 amino acids (IDLG) but failed to conserve the terminal half of the motif (TTXS). Finally, as noted by Lee-Yoon et al. (13) for hsp110 but not shown in Fig. 4, the extracellular domain of the sea urchin sperm receptor (16, 17), and two ORFs from yeast known as SSE1 and SSE2 (18, 19) are also homologous to Osp94.

Stress-induced mRNA Expression—An analysis of the time-dependent response of Osp94 to hyperosmotic NaCl (Fig. 6) showed that mRNA levels were transiently elevated. mRNA expression was slightly increased at 3 h, maximally increased at 12–24 h and returned to normal by 72 h despite ongoing exposure to hyperosmolar NaCl. GAPDH mRNA levels were unchanged by hyperosmotic NaCl during this same period.

Since Osp94 is structurally related to the hsp70 superfamily it was of interest to determine whether Osp94 was also inducible by heat shock. As shown in Fig. 7, heat shock increased Osp94 expression in a manner similar to that seen with other heat stress inducible mRNAs. mRNA expression increased within 1 h of heat shock, was maximal at 3 h, and returned to approximately control levels at 24 h. Not shown, tunicamycin, a well known inducer of BiP expression failed to elicit an increase in Osp94 expression. Thus, Osp94 is a heat shock-inducible gene akin to hsp70.

Kidney Expression—To evaluate a potential in vivo role for Osp94, we examined mRNA expression in mouse kidney (Fig. 8). Osp94 was highly expressed in mouse kidney and showed a pattern of increasing expression from cortex to inner medulla.
that correlates with the well known renal corticomedullary osmolality gradient (26). Moreover, mice subjected to a dehydration protocol in which the renal inner medullary osmolality is known to increase (26) show enhanced expression of Osp94 in inner medulla. Not shown, extremely low levels of Osp94 mRNA expression were evident in brain, heart, liver, and intestine and they appeared unchanged with dehydration. Thus, Osp94 is expressed in kidney in vivo and renal inner medullary mRNA expression level responds to a hyperosmotic challenge.

**DISCUSSION**

Osp94 is an hyperosmotic and heat stress-inducible member of the recently described hsp110/SSE gene subfamily (13). The members of this subfamily have emerged as a relatively large and evolutionarily conserved group that includes structurally related genes from mammals, yeast, C. elegans, and sea urchin. With the exception of the sea urchin sperm receptor, the members of this subfamily have no known functions but their structural similarities to hsp70, hsc70, and BiP provide indirect evidence they function as molecular chaperones.

Osp94, like hsp110 and hsp70RY, possesses two structurally distinct domains. The N-terminal half contains a putative ATP-binding domain that is characteristic of other members of this gene subfamily. Moreover, like hsp70, hsc70, and BiP (27, 28), the C-terminal portion of Osp94 contains a putative peptide-binding domain. Within this region of the hsp70 family of molecules a variable, substrate recognition domain has been characterized (10, 27, 29) whose tertiary structure shows similarity to the variable peptide-binding domain of the antigen

![Fig. 4. Deduced amino acid sequence of Osp94 (accession no. U23921) and its alignment with two homologous proteins, hsp110 (13) (Z47807) and hsp70RY (14) (L12723). Shaded areas indicate amino acid identities among the three proteins. Overlined amino acids indicate the putative five motifs of the ATP binding domain and correspond, in order, to the segments known as phosphate 1, connect 1, phosphate 2, adenosine, and connect 2 (23).](image-url)
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Fig. 5. Tree of homology of several mammalian hsp70 and hsp10 family members based on the unweighted pair group method with arithmetic mean algorithm. Deduced amino acid sequences of mouse Osp94 (GenBankTM accession no. U23921), hamster hsp11 (Z47807), human hsp70R (L12723), mouse hsp70 (M35021), mouse hsc70 (M19411), and mouse BiP (P20029; Swiss-Prot) were compared. The lengths of the horizontal lines are proportional to the estimated genetic distances. The numbers indicate the average percentage identity among different proteins beyond the branch point.

Fig. 6. Time course of Osp94 mRNA expression during hyperosmotic stress. Total RNA was isolated from ml MCD3 cells exposed to isosomolar (−) or hyperosmolar NaCl (+) for 0.5 to 72 h. Upper figure shows Osp94 mRNA expression was transiently increased with maximal expression observed at 12–24 h. Lower figure shows GAPDH mRNA level was relatively unchanged. The larger 11.2-kb Osp94 transcript observed in Fig. 1B is not shown.

Fig. 7. Time course of Osp94 mRNA expression during heat stress. RNA was isolated from ml MCD3 cells exposed to normal (37°C) or elevated (42°C) temperatures for 0.5 to 24 h. Northern analysis (10 μg of total RNA) shows increasing Osp94 expression from cortex to inner medulla. Dehydrated mice expressed elevated levels of Osp94 mRNA in inner medulla. GAPDH mRNA levels were relatively unchanged.

Fig. 8. Expression of Osp94 mRNA in kidneys from control and dehydrated mice. Control mice were provided ad libitum access to water, and dehydrated mice were water-restricted for 24 h to induce an increase in renal inner medullary osmolality. Total RNA was prepared from three regions of the kidney: cortex, outer medulla, and inner medulla. Northern analysis (10 μg of total RNA) shows increasing Osp94 expression from cortex to inner medulla. Dehydrated mice expressed elevated levels of Osp94 mRNA in inner medulla. GAPDH mRNA levels were relatively unchanged.

Osp94 mRNA expression was greatly increased with maximal expression observed at 3 h. Lower figure shows GAPDH mRNA level was relatively unchanged.

hsp70 expression (9). Moreover, the transient expression of Osp94 mRNA correlates with the known rate of accumulation of protein-stabilizing organic osmolytes in renal epithelial cells, suggesting that the stress proteins serve a cytoprotective role during the period of high intracellular salt stress that precedes organic osmolyte accumulation (2, 31).

Heat shock also induced mRNA expression indicating Osp94 is a heat shock-inducible gene like hsp70. Similarly, hsp110 was shown to be induced by heat shock in CHO cells (13), whereas the response of hsp70RY to heat stress is unknown. The failure of tunicamycin to induce Osp94 expression distinguishes it from BiP and other endoplasmic reticulum localized stress proteins suggesting Osp94 is not associated with endoplasmic reticulum-mediated functions. Hsp110 was found to be localized primarily in the cytoplasm and the periphery of nuclei (13). The subcellular localization of Osp94 is unknown at this time and will undoubtedly be an issue of significant interest in the future. Moreover, whether other stressors can induce expression of Osp94 and other members of the hsp110/SSE subfamily remains to be explored.

Analysis of renal expression of Osp94 provided direct evidence that this gene is responsive to osmotic stress in vivo. The renal urine concentrating mechanism requires the inner medulla to be hyperosmotic. In rodents, such as mouse, inner medullary osmolality is approximately 1000 mOsm under control conditions, and with water restriction osmolality rises severalfold over the course of 1–3 days (26). This hyperosmotic inner medullary milieu is comprised in large part of a high concentration of NaCl. In control mice, Osp94 mRNA expression showed a cortical-medullary gradient profile that paralleled the known renal osmolality gradient. Moreover, with 24 h

presenting major histocompatibility complex class I proteins (30). Two portions of this C-terminal region within Osp94 (Val497–Gln594 and Lys 707–Asp838) contain nonconserved amino acid sequences that failed to show significant homology to any proteins, including hsp90, in the sequence data bases. The functional significance of the C-terminal domain including identification of potential peptide ligands which may be bound by Osp94 remains to be elucidated and will likely reveal the functional role of Osp94 and other members of the hsp110/SSE subfamily.

In vitro translation of Osp94 showed it migrated on SDS-PAGE with an apparent size of 105–110 kDa, somewhat greater than its calculated molecular mass of 94 kDa. This observation is consistent with the fact that hsp110 has a calculated molecular mass of 96,042 Da which is less than the 110-kDa size observed on Western analysis (13).

Northern analysis showed three distinct Osp94 transcripts whose expression levels varied in synchrony. The 4.1-kb transcript contains a larger 3'UTR indicative of an alternative polyadenylation site. The significance of the minor 11.2-kb transcript remains to be elucidated, but it is reasonable to hypothesize that it is derived from the same gene and encodes the same protein but contains a larger 3'UTR. At present, there is no evidence of alternative splicing within the coding region of Osp94.

Northern analysis delineated the response of Osp94 to hyperosmotic stress. In ml MCD3 cells, hyperosmotic NaCl transiently increased Osp94 expression. This response is similar to that observed previously for hsp70 (5, 6). Although the mechanism of induction is unknown, it is noteworthy that this level of hyperosmotic stress is known to suppress protein synthesis (1), and disruption of protein synthesis is a known stimulus of
of water restriction, Osp94 expression was significantly elevated in renal inner medulla consistent with its activation by hyperosmotic stress.

In conclusion, Osp94 is a new member of the hsp110/SSE gene subfamily. Osp94 is inducible by hyperosmotic stress and heat shock. The physiological and biochemical functions of Osp94 are unknown but it, like other members of the larger hsp70 superfamily of genes, likely acts as a molecular chaperone.

REFERENCES

1. Cohen, D. M., and Gullans, S. R. (1993) Am. J. Physiol. 264, F601–F607
2. Burg, M. B. (1995) Am. J. Physiol. 268, F983–F996
3. Garcia-Perez, A., and Burg, M. B. (1991) Physiol. Rev. 71, 1081–1113
4. Burg, M. B., and Garcia-Perez, A. (1992) J. Am. Soc. Nephrol. 3, 121–127
5. Cohen, D. M., Wasserman, J. C., and Gullans, S. R. (1991) Am. J. Physiol. 261, C994–C1001
6. Shekh-Hamad, D., Garcia-Perez, A., Ferraris, J. D., Peters, E. M., and Burg, M. B. (1994) Am. J. Physiol. 267, F28–F34
7. Tanaka, K., Jay, G., and Isselbacher, K. (1988) Biochim. Biophys. Acta 950, 138–146
8. Dasgupta, S., Hohman, T., and Carper, D. (1992) Exp. Eye Res. 54, 461–470
9. Welch, W. J. (1992) Physiol. Rev. 72, 1063–1081
10. Gething, M., and Sambrook, J. (1992) Nature 355, 33–45
11. Becker, J., and Craig, E. A. (1994) Eur. J. Biochem. 219, 11–23
12. Noer, L., and Scharf, K. D. (1991) in Heat Shock Response (Noer, L., ed), pp. 41-128, CRC Press, Boca Raton, FL
13. Lee-Yoon, D., Easton, D., Murawski, M., Burd, R., and Subjeck, J. R. (1995) J. Biol. Chem. 270, 15725–15733
14. Fahdallah, D. M., Cherif, D., Deliagi, K., and Arnaout, M. A. (1993) J. Immun. 151, 810–813
15. Foltz, K. R., Partin, J. S., and Lennarz, W. J. (1993) Science 259, 1421–1425
16. Suston, J. Du, Z., Thomas, W., Wilson, R., Hillier, L., Staden, R., Halloran, N., Green, P., Thierry-Mieg, J., Qui, L., Dear, S., Coulson, A., Craxton, M., Durbin, R., Berks, M., Metzstein, M., Hawkins, T., Ainscough, R., and Waterston, R. (1992) Nature 356, 37–41
17. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copey, T., Cooper, J., Coulson, A., Craxton, M., S., Du, Z., Durbin, R., Favello, A., Fraser, A., Fulton, A., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Lasloller, M., Latrelle, P., Lightning, J., Mortimore, B., O’Callaghan, M., Parsons, J., Percy, C., Riffken, L., Roepr, A., Saunders, D., Shown, Keen, R., Sims, M., Smaldon, N., Smith, A., Smith, M., Sonnhammer, E., Staden, R., Suston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson, L., Wilkinson-Sproat, J., and Wohldman, P. (1994) Nature 368, 32–38
18. Mukai, H., Kuno, T., Tanaka, H., Hirata, D., Miyakawa, T., and Tanaka, C. (1993) Gene (Amst.) 132, 57–66
19. Shirayama, M., Kawakami, K., Mitsui, Y., Tanaka, K., and Toh-E, A. (1993) Mol. & Gen. Genet. 240, 323–332
20. Rauchman, M. I., Despré, E., Nigam, S., and Gullans, S. R. (1993) Am. J. Physiol. 265, F416–F424
21. Liang, P., and Pardee, A. B. (1992) Science 257, 967–971
22. Nakai, K., and Kanehisa, M. (1992) Genomics 14, 89–911
23. Bork, P., Sander, C., and Valenda, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7290–7294
24. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
25. Parfett, C. L. J., Hofbauer, R., Brudynski, K., Edwards, D. R., and Denhardt, D. T. (1989) Gene (Amst.) 62, 291–303
26. Yancey, P. H. (1988) J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 158, 369–380
27. Milarski, R. L., and Morimoto, R. I. (1989) J. Biol. Chem. 264, 1947–1962
28. Wang, T.-F., Chang, J., and Wang, C. (1993) J. Biol. Chem. 268, 26049–26051
29. Chappell, T. G., Konforti, B. B., Schmid, S. L., and Rothman, J. E. (1987) J. Biol. Chem. 262, 746–751
30. Ripplmann, F., Taylor, W. R., Rothbard, J. B., and Green, N. M. (1991) EMBO J. 10, 1053–1059
31. Cohen, D. M., and Gullans, S. R. (1993) in Cellular and Molecular Physiology of Cell Volume Regulation (Strange, K., ed), pp. 363–372, CRC Press, Boca Raton, FL