Biochemical and Biophysical Research Communications **262,** 231–236 (1999)
Article ID bbrc.1999.1079, available online at http://www.idealibrary.com on IDEAL®

Involvement of Receptor Aggregation and Reactive Oxygen Species in Osmotic Stress-Induced Syk Activation in B Cells

Suofu Qin, Junyi Ding, Tomoko Takano, and Hirohei Yamamura
Department of Biochemistry, Kobe University School of Medicine, Chuo-ku, Kobe 650-0017, Japan

Received June 21, 1999

Syk has been shown to be activated by osmotic stress, however, the mechanisms involved are largely unknown. In this study, we demonstrated that cell shrinkage, rather than osmolarity, was responsible for osmotic stress-induced Syk activation. Osmotic stress-induced Syk activation depended partly upon aggregation of surface receptors. Moreover, intracellular reactive oxygen species were involved in mediating osmotic stress-induced Syk activation, with osmotic stress-induced Syk activation being inhibited by the pretreatment of cells with *N*-acetyl-cysteine and reduced glutathione. When cells were treated with the combination of sodium chloride and hydrogen peroxide, there was a synergistic activation of Syk. In conclusion, osmotic stress-induced Syk activation required suramin-inhibitable surface receptor aggregation and accumulation of intracellular reactive oxygen species.

Key Words: **osmotic stress; reactive oxygen species; protein-tyrosine kinase; Syk.**

Protein-tyrosine kinases (PTKs) play crucial roles in a wide variety of cellular responses, including cell activation, proliferation and differentiation (1). In addition to growth factors and cytokines, which stimulate receptor intrinsic PTK activities or receptor-coupled non-receptor PTKs, following binding to their cognate receptors (2), extracellular stress stimuli such as ionizing radiation, ultraviolet radiation, hydrogen peroxide and genotoxic agents, can also activate PTKs, including Src- and Syk-family PTKs (3–7). Here, we are particularly interested in Syk, a non-receptor PTK that was previously cloned in our laboratory (8). Syk is strongly activated following B cell receptor engagement (9, 10), by hydrogen peroxide (11–13) and by osmotic stress (13, 14). Both oxidative and osmotic stress also elicits increased tyrosine phosphorylation of cellular proteins, activation of c-Jun N-terminal kinases (JNKs), calcium mobilization and cell apoptosis in chicken DT40 B cells (13, 14). However, genetic studies utilizing Syk-negative cells demonstrated clearly that Syk plays distinctive roles in oxidative and osmotic stress signaling. Oxidative stress induces a Syk-dependent increase in intracellular calcium concentration, JNK activation, and tyrosine phosphorylation of cellular proteins, but osmotic does not (13, 15). Syk appears to function as an inhibitor of osmotic stress-induced apoptosis, but has little function in oxidative stress-induced apoptosis (14). Different functions of Syk might be accounted for partly by differential regulation of Syk activation in response to osmotic and oxidative stress. While Syk activation was generated by the combination of both Syk activity and additional PTK activity, Syk activity and additional PTK activity were responsible for the majority of oxidative stress- and osmotic stress-induced Syk tyrosine phosphorylation, respectively (15). Point mutation within C-terminal Src homology domain of Syk enhanced Syk activity following osmotic, but not oxidative stress treatment (15).

The interaction of tyrosine-phosphorylated receptor subunits with the tandem SH2 domains of Syk has been implicated as a major mechanism for Syk activation by antigen receptors in hematopoietic cells (16–19). However, the mechanisms by which stress-derived signals activate Syk remain unaddressed. Osmotic stress was reported to induce the clustering of growth factor and cytokine receptors, which were a prerequisite for the activation of these receptors. The activation of these receptors was essential for subsequent osmotic response (20). Several groups recently demonstrated that rather than the change of extra and/or intracellular tonicity, it was membrane deformation that was responsible for osmotic stress-induced tyrosine phosphorylation and other biochemical events (21–23). In addition, induction in intracellular reactive oxygen

Abbreviations used: PTK, protein-tyrosine kinase; JNK, c-Jun N-terminal kinase; ROS, reactive oxygen species; NAC, *N*-acetyl-cysteine; GSH, reduced glutathione.

0006-291X/99 $30.00

species (ROS) has been demonstrated to be an important prerequisite for ligand-dependent cell activation and signal transduction (24–30). Osmotic stress was reported to induce the accumulation of intracellular ROS (31) and intracellular ROS were indicated to be required for the regulation of certain osmotic response (32). To explore the nature of how the signals derived from osmotic stress activate Syk in B cells, we applied suramin, an inhibitor of cell surface receptor aggregation, and ROS scavengers such as *N*-acetyl-cysteine (NAC) and the reduced form of glutathione (GSH), to dissect the possible events involved in osmotic stress-induced Syk activation. In comparison, hydrogen peroxide that itself is one of the ROS and Syk activator is also used as a stimulator in this study. We reported here that osmotic stress-induced Syk activation partly depended on cell surface receptor aggregation and intracellular ROS generation.

MATERIALS AND METHODS

Materials and chemicals. RPMI 1640 powder was purchased from GIBCO Inc. Protein A was from Calbiochem Corporation. Hydrogen peroxide, sodium chloride, potassium chloride and sucrose were from Wako Pure Chemicals. Anti-phosphotyrosine antibody (4G10) was from Upstate Biotechnology Inc. Nystatin, reduced glutathione (GSH), *N*-acetyl-cysteine (NAC) and suramin were from Sigma. Enhanced chemiluminescence reagents were from Dupont.

Cell culture and harvest. Establishment of Syk-negative DT40 cells expressing porcine Syk (designated Syk-/Syk), was performed as described previously (33). Syk-/Syk DT40 cells and human Raji B cells were maintained in RPMI-1640 medium, supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified 95% air/CO_2 atmosphere. For experiments, cells were collected by centrifugation as previously described (12). Cells were stimulated by hydrogen peroxide (oxidative stress) or sodium chloride (osmotic stress) at 37°C.

Cell permeabilization by nystatin. For the experiments required cells to be permeabilized, cells were resuspended in isotonic NaCl buffer (140 mM NaCl, 5 mM KCl, 10 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Hepes, pH 7.5), or isotonic KCl buffer (145 mM KCl, 10 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Hepes, pH 7.5), or iso-osmotic sucrose buffer (280 mM sucrose, 5 mM KCl, 10 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Hepes, pH 7.5), and then were permeabilized with 500 units/ml nystatin at 37°C prior to stimulation. When required, media were made hypertonic to the indicated level in the text by the addition of NaCl or KCl or sucrose to the respective isotonic buffer.

Preparation of cell extracts. Stimulated cells ($5 \sim 10 \times 10^6$ cells/ml) were lysed in ice-cold lysis buffer (5 mM EDTA, 150 mM NaCl, 1% Triton X-100, 100 μM Na_3VO_4, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 50 mM Tris, pH 7.4) following a short centrifugation step. Lysates were clarified by centrifugation at 16,000 $\times$ g for 15 min at 4°C.

Immunoblot analysis. Cell extracts or immunoprecipitates were resolved on SDS-PAGE, transferred electrophoretically onto PVDF membranes, and then immunoblotted with the indicated antibodies. Immunoreactive proteins were visualized by enhanced chemiluminescence.

RESULTS

Cell shrinkage, but not osmolarity, induced Syk activation in Raji B cells. Osmotic stress activated Syk and induced tyrosine phosphorylation on cellular proteins (15, 21, 22). The activation of Syk by osmotic stress may be due to a change in the extra- and/or intracellular total osmotic activity or an alteration in the cell volume. To dissect the imposed changes in tonicity from the changes in the cell volume, we applied hypertonicity, while preventing cell shrinkage by using the non-selective monovalent ionophore, nystatin, a pore-forming molecule that allows the passage of small monovalent ions across the plasma membrane (34). Pretreatment of cells with nystatin has been reported to effectively block cell shrinkage and tyrosine phosphorylation of cellular proteins induced by osmotic sodium chloride or potassium chloride medium (21, 22). Nystatin inhibited tyrosine phosphorylation of cellular proteins induced by osmotic media, such as a hypertonic sodium medium (Fig. 1A). Thus we used this approach to explore the effect of cell shrinkage on osmotic stress-induced Syk activation. As Syk is activated by tyrosine phosphorylation following antigen receptor engagement or hydrogen peroxide treatment, the phosphorylation status of this protein was used as an indicator of its activation state, as previously described (11, 35, 36). Figure 1B illustrated that treatment of Raji cells with nystatin did not affect the status of Syk tyrosine phosphorylation, but substantially reduced the level of osmotic stress-induced Syk activation, regardless of the solute used (Fig. 1B). When the extra- and intracellular osmolarity was increased by urea, a rapidly permeating solute, Syk tyrosine phosphorylation in cells exposed to osmotic urea (0.2 or 0.3 M) was similar to that of cells maintained in isotonic buffer (Fig. 1B and data not shown), clearly indicating that an increase in extra- and intracellular tonicity was unable to activate Syk.

To further ascertain that cell shrinkage, rather than osmolarity, was responsible for Syk activation, we took the second approach of enhancing cell shrinkage, without further increasing extra- and intracellular osmolarity. While the extracellular sucrose was unable to diffuse through the nystatin pores, intracellular monovalent ions easily diffused out of the cells. When the same dose of sucrose was added to the cells treated with and without nystatin, osmotic sucrose exposure caused a further reduction in the cell volume in nystatin-treated cells compared to untreated cells (21, 22). Figure 1A and 1B showed that addition of sucrose to the cells suspended in iso-osmotic sucrose medium caused moderate tyrosine phosphorylation of cellular proteins and a weaker Syk activation. When the same dose of sucrose was added to the nystatin-permeabilized cells, stronger Syk activation and tyrosine phosphorylation of cellular proteins were elic-

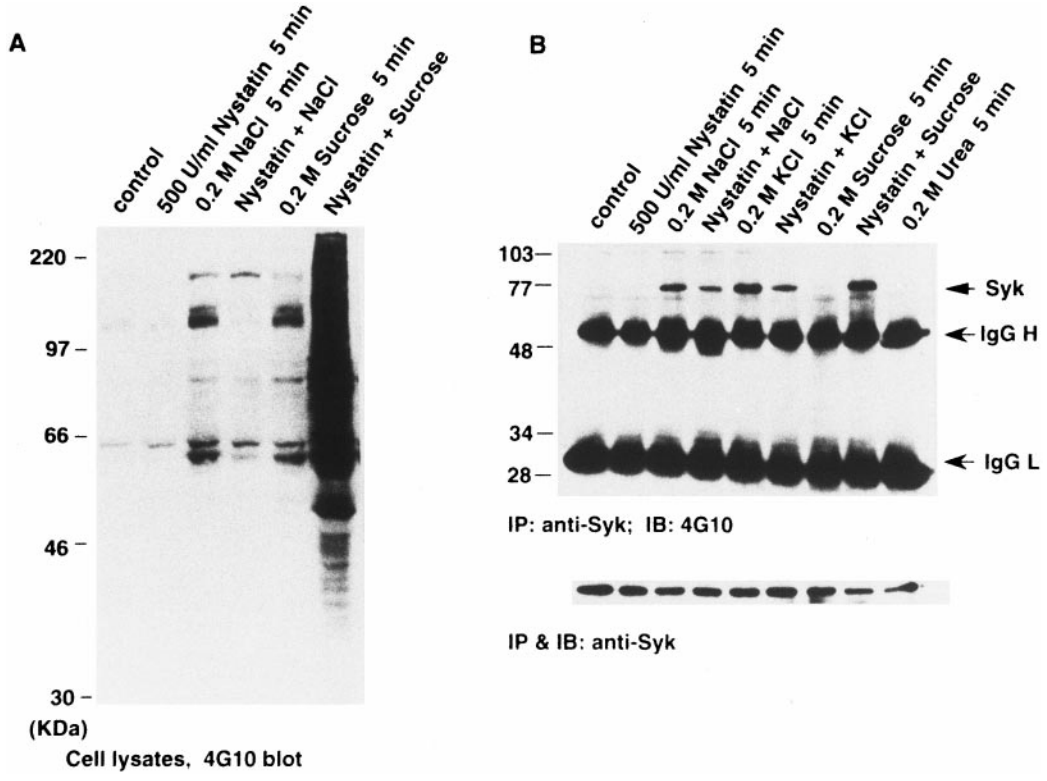

FIG. 1. Syk activation and tyrosine phosphorylation of cellular proteins induced by osmotic stress in human Raji B cells. (A) Anti-phosphotyrosine immunoblot of cell lysates from Raji cells. Cells were pretreated with or without 500 units/ml nystatin for 5 min and then were subjected to osmotic stress treatment (an additional 0.2 M sodium chloride, or 0.2 M sucrose). Cell lysates were separated by SDS-PAGE, transferred to PVDF membrane, and immunoblotted with an anti-phosphotyrosine antibody. (B) Anti-phosphotyrosine immunoblot (top) and anti-Syk immunoblot (bottom) of anti-Syk immunoprecipitates from Raji cells following osmotic stress treatment for 5 min. IP, immunoprecipitation; IB, immunoblot. The positions of the molecular-mass markers are shown on the left (kDa).

ited (Fig. 1A and 1B). Together these results demonstrated that cell shrinkage activated Syk, regardless of the osmolarity.

Surface receptor aggregation was involved in osmotic stress-, but not oxidative stress-induced Syk activation. Cells may perceive a change in their volume by sensing macromolecular crowding (37). One form of crowding is the aggregation of cell surface receptors. It has been extensively shown that such clustering of receptors is crucial for their activation (38). Osmotic shrinkage of HeLa cells was reported to induce clustering of interleukin-1, epidermal growth factor, and tumor necrosis factor receptors in the absence of their ligands (20). Oxidizing agents also can trigger receptor clustering, to some extent, through sulfhydryl oxidation. Therefore, we explored the role of receptor clustering in the initiation of osmotic and oxidative stress-induced Syk activation. Suramin is known to block both ligand- and nonligand-induced receptor aggregations, thereby abolishing receptor-mediated signal transduction (39). As shown in Fig. 2, suramin inhibited osmotic stress-induced tyrosine phosphorylation of Syk, inferring the involvement of surface receptor aggregation in osmotic stress-induced Syk activation. Conversely, oxidative stress-induced tyrosine phosphorylation of Syk was not affected by suramin pretreatment. Thus, oxidative stress appeared to bypass surface receptor aggregation in triggering Syk activation.

Inhibition of osmotic stress-induced Syk activation by antioxidants. We next investigated the nature of the signals derived from sodium chloride that initiates the activation of Syk. Here, we examined whether the activation of Syk by sodium chloride and hydrogen peroxide was sensitive to NAC or GSH, which would suggest an involvement of ROS. Raji cells were preincubated in the absence or presence of NAC or GSH, and then stimulated with sodium chloride or hydrogen peroxide. Enhancing the cellular antioxidant potential by the addition of 2 mM of either NAC or GSH abolished the capability of both sodium chloride and hydrogen peroxide to activate Syk (Fig. 3A, top) and to induce the increase in tyrosine phosphorylation of cellular proteins (data not shown). These results indicated that ROS mediated both osmotic and oxidative stress-initiated Syk activation in Raji cells. Somewhat to our surprise, by increasing the NAC concentration to 6 mM, this inhibitory effect disappeared. Reprobing

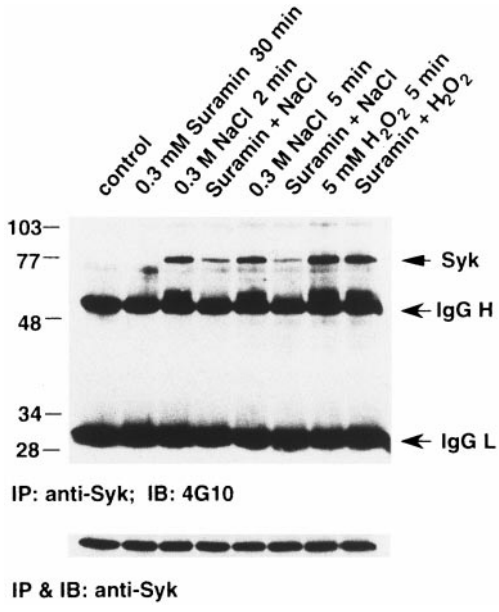

FIG. 2. Effect of suramin on osmotic and oxidative stress-induced Syk activation. Cells were preincubated with or without 0.3 mM suramin for 30 min and then stimulated by an additional 0.3 M sodium chloride or 5 mM hydrogen peroxide for indicated time points. Anti-Syk immunoprecipitates from Raji cell lysates were immunoblotted with an anti-phosphotyrosine immunoblot (top) and anti-Syk immunoblot (bottom).

the same membrane with anti-Syk antibody revealed that a similar amount of Syk was immunoprecipitated in all samples except those treated with 6 mM NAC, which appeared to severely reduce the recovery of Syk by the anti-Syk antibody (Fig. 3A, bottom). To look for the generality of the requirement of ROS for osmotic and oxidative stress-induced Syk activation, we investigated whether NAC can block oxidative and osmotic stress-induced Syk activation in other cell systems. Syk-negative DT40 cells expressing porcine Syk was used since we have a good antibody raised against porcine Syk. As in the case of Raji cells, similar results were obtained in DT40 chicken B cells (Fig. 3B).

Synergistic effect of sodium chloride and hydrogen peroxide on Syk activation. That osmotic stress-stimulated Syk activation could be inhibited by the pretreatment of cells with antioxidants indicated that osmotic stress stimulated an increase in intracellular ROS. Thus, we reasoned that the combination of sodium chloride and hydrogen peroxide could activate Syk in an additive or synergistic manner. To test this possibility, we carried out immunoblot analysis with antiphosphotyrosine antibody on Syk immunoprecipitated from Raji cell lysates treated with sodium chloride and/or hydrogen peroxide. Syk was only moderately activated by treatment with either sodium chloride or hydrogen peroxide (Fig. 4). Treatment with both sodium chloride and hydrogen peroxide strongly activated Syk, to a much higher extent than that seen with sodium chloride or hydrogen peroxide alone, demonstrating that sodium chloride and hydrogen peroxide activated Syk in a synergistic manner. When sodium chloride was replaced with potassium chloride, the same result was obtained (Fig. 4).

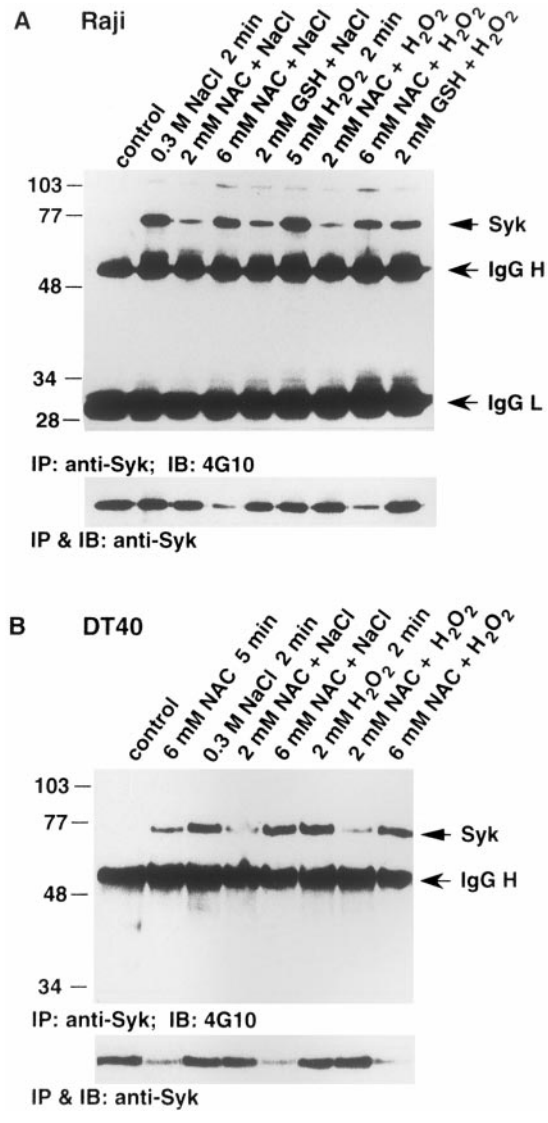

FIG. 3. Inhibition of osmotic and oxidative stress-induced Syk activation by antioxidants. (A) Involvement of reactive oxygen species in cell shrinkage and oxidative stress-induced Syk activation in Raji cells. Cells were preincubated with the indicated doses of *N*-acetyl cysteine (NAC) or reduced glutathione (GSH) for 5 min and then stimulated for 2 min by an additional 0.3 M sodium chloride or 5 mM hydrogen peroxide. Anti-Syk immunoprecipitates were immunoblotted with an anti-phosphotyrosine antibody (top) and anti-Syk antibody (bottom). (B) Involvement of reactive oxygen species in osmotic and oxidative stress-induced Syk activation in DT40 cells. Syk-negative DT40 cells expressing porcine Syk were incubated with NAC for 5 min and then stimulated for 2 min by an additional 0.3 M sodium chloride or 2 mM hydrogen peroxide.

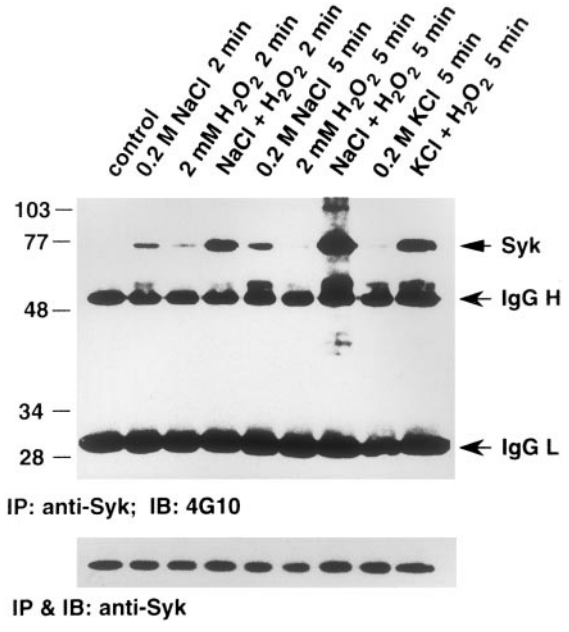

FIG. 4. Synergistic effect of osmotic and oxidative stress on Syk activation. Raji cells were treated with 0.2 M sodium chloride (or potassium chloride) and/or 2 mM hydrogen peroxide for 2 or 5 min. Anti-Syk immunoprecipitates were immunoblotted with an anti-phosphotyrosine antibody (top) and anti-Syk antibody (bottom).

DISCUSSION

Syk plays a crucial role in platelet activation, B cell development, and hematopoietic cell signaling (9, 10, 19, 40–42). In addition, two stress stimuli, osmotic and oxidative stress, activate Syk in B cells (11–14). Genetic studies further reveal that Syk plays distinctive roles in osmotic and oxidative stress signaling (13–15). However, the intermediate steps involved in the transduction of activating signals derived from osmotic and oxidative stress to Syk, are largely unknown. Here we have provided evidence that the triggering factor responsible for osmotic stress-induced Syk activation is not an increase in extra- or intracellular osmotic concentration *per se*, but rather a decrease in cell volume. Cell shrinkage appears to be the prerequisite for osmotic stress-induced Syk activation. This conclusion was supported by the following evidence. Syk activation could be reduced by the inhibition of cell shrinkage with nystatin at constant osmolarity and ionic strength. On the other hand, while maintaining constant osmolarity, shrinkage of nystatin-permeabilized cells with sucrose caused a marked increase in Syk tyrosine phosphorylation (Fig. 1B). Moreover, in the absence of significant cell volume changes, increase in extra- and intracellular osmolarity with urea, a rapidly permeating solute, failed to activate Syk. It is noteworthy that the incomplete inhibition of Syk activation in nystatin-permeabilized cells by sodium chloride or potassium chloride, may have been caused by a transient cell shrinkage that likely happened when the osmolarity of the medium was raised, as water efflux from the cells treated with nystatin may have preceded entry and equilibration of sodium chloride into the cells.

It has been hypothesized that cells perceive their volume by sensing macromolecular crowding. One form of crowding may be the aggregation of surface receptors as recently reported (20). Oxidizing agents also can trigger the aggregation of many growth factor and cytokine receptors that contain cysteine-rich motifs (38, 43). Cell surface receptor aggregation has already been demonstrated to be required for osmotic stress-induced JNK activation (20) and hydrogen peroxide-stimulated extracellular signal regulated kinase (ERK) activation (44). Pretreatment of B cells with suramin, an inhibitor of cell surface receptor aggregation, indeed inhibited osmotic stress-induced Syk activation (Fig. 2). In B cells, crosslinking of B cell receptors by an anti-IgM antibody (9, 10) or oligomerizing cell-surface glycoproteins by lectins (45, 46), led to Syk activation. It is tempting to speculate that shrinkage of B cells triggers the activation of Syk through clustering of B cell antigen receptors and/or other tyrosine kinase (associated) receptors. In contrast, oxidative stress-induced Syk activation appeared to bypass the requirement of suramin-inhibitable aggregation of cell surface receptors.

A growing body of evidence demonstrated that intracellular ROS are important second messengers in mediating the signal transduction pathways elicited by various agonists (24–30). Involvement of ROS in sodium chloride-induced Syk activation was tested by pretreating the cells with the widely used antioxidant, NAC which can detoxify ROS either indirectly or directly. NAC significantly reduced sodium chloride-induced Syk activation (Fig. 3). GSH also can inhibit Syk activation. The sensitivity of sodium chloride-induced Syk activation to NAC and GSH clearly indicated that Syk activation by sodium chloride was elicited by ROS produced in response to osmotic stress. It was unexpected that sodium chloride- and hydrogen peroxide-induced Syk activation was hardly inhibited by high dose of NAC (Fig. 3). At present, we have no idea about this phenotype, however, it is possible that high concentration of NAC itself is one kind of stresses which can activate Syk.

ROS were prerequisite, at least in part, for osmotic stress-induced Syk activation. Osmotic stress needed surface receptor aggregation to activate Syk, but oxidative stress-induced Syk activation bypassed this requirement. Thus, we reasoned that the combined treatment of cells by sodium chloride and hydrogen peroxide may activate Syk in an additive or synergistic manner. Immunoblot analysis of anti-Syk immunoprecipitates with anti-phosphotyrosine antibody revealed that sodium chloride synergized with hydrogen peroxide to activate Syk. The same result was also observed when

sodium chloride was replaced by potassium chloride, suggesting that ROS generation might account for the synergistic activation of Syk by oxidative and osmotic stress.

In a word, cell shrinkage rather than osmolarity was responsible for osmotic stress-induced Syk activation. Accumulation of intracellular ROS was a prerequisite for Syk activation elicited by both osmotic and oxidative stress, two stress stimuli that synergize in relation to Syk activation

ACKNOWLEDGMENTS

We thank Dr. Tomohiro Kurosaki for providing Syk-negative DT40 cells expressing porcine Syk and Dr. Allison Stewart for her help in manuscript preparation. This study was supported, in parts, by Grants-in-Aid for Science Research from the Ministry of Education, Science, Sports, and Culture of Japan for General Scientific Research, Scientific Research on Priority Areas, and Yamanouchi Foundation for Research on Metabolic Disorders.

REFERENCES

1. Ullrich, A., and Schlessinger, J. (1990) *Cell* **61,** 203–212.
2. Taniguchi, T. (1995) *Science* **268,** 251–255.
3. Brumell, J. H., Burkhardt, A. L., Bolen, J. B., and Grinstein, S. (1996) *J. Biol. Chem.* **271,** 1455–1461.
4. Hardwick, J. S., and Sefton, B. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4527–4531.
5. Kharbanda, S., Yuan, Z. M., Rubin, E., Weichselbaum, R., and Kufe, D. W. (1994) *J. Biol. Chem.* **269,** 20739–20743.
6. Kharbanda, S., Yuan, Z. M., Taneja, N., Weichselbaum, R., and Kufe, D. W. (1994) *Oncogene* **9,** 3005–3011.
7. Schieven, G. L., Kirihara, J. M., Myers, D. E., Ledbetter, J. A., and Uckun, F. M. (1993) *Blood* **82,** 1212–1220.
8. Taniguchi, T., Kobayashi, T., Kondo, J., Takahashi, K., Nakamura, K., Suzuki, J., Nagai, K., Yamada, T., Nakamura, S., and Yamamura, H. (1991) *J. Biol. Chem.* **266,** 15790–15796.
9. Hutchcroft, J. E., Harrison, M. L., and Geahlen, R. L. (1992) *J. Biol. Chem.* **267,** 8613–8619.
10. Yamada, T., Taniguchi, T., Yang, C., Yasue, S., Saito, H., and Yamamura, H. (1993) *Eur. J. Biochem.* **213,** 455–459.
11. Schieven, G. L., Kirihara, J. M., Burg, D. L., Geahlen, R. L., and Ledbetter, J. A. (1993) *J. Biol. Chem.* **268,** 16688–16692.
12. Qin, S., Inazu, T., Takata, M., Kurosaki, T., Homma, Y., and Yamamura, H. (1996) *Eur. J. Biochem.* **236,** 443–449.
13. Qin, S., Minami, Y., Hibi, M., Kurosaki, T., and Yamamura, H. (1997) *J. Biol. Chem.* **272,** 2098–2103.
14. Qin, S., Minami, Y., Kurosaki, T., and Yamamura, H. (1997) *J. Biol. Chem.* **272,** 17994–17999.
15. Qin, S., Kurosaki, T., and Yamamura, H. (1998) *Biochemistry* **37,** 5481–5486.
16. Rowley, R. B., Burkhard, A. L., Chao, H. G., Matsueda, G. R., and Bolen, J. B. (1995) *J. Biol. Chem.* **270,** 11590–11594.
17. Shiue, L., Zoller, M. J., and Brugge, J. S. (1995) *J. Biol. Chem.* **270,** 10498–10502.
18. Scharenberg, A. M., Lin, S., Cuenod, B., Yamamura, H., and Kinet, J. P. (1995) *EMBO J.* **14,** 3385–3394.
19. Lin, S., Cicala, C., Scharenberg, A. M., and Kinet, J. P. (1996) *Cell* **85,** 985–995.
20. Rosette, C., and Karin, M. (1996) *Science* **274,** 1194–1197.
21. Krump, E., Nikitas, K., and Grinstein, S. (1997) *J. Biol. Chem.* **272,** 17303–17311.
22. Szaszi, K., Buday, L., and Kapus, A. (1997) *J. Biol. Chem.* **272,** 16670–16678.
23. Sadoshima, J., Qiu, Z., Morgan, J. P., and Izumo, S. (1996) *EMBO J.* **15,** 5535–5546.
24. Busciglio, J., and Yankner, B. A. (1995) *Nature* **378,** 776–779.
25. Johnson, T. M., Yu, Z. X., Ferrans, V. J., Lowenstein, R. A., and Finkel, T. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11848–11852.
26. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) *Science* **270,** 296–299.
27. Irani, K., Xia, Y., Zweier, J. L., Sollott, S. J., Der, C. J., Fearon, E. R., Sundaresan, M., Finkel, T., and Goldschmidt-Clermont, P. J. (1997) *Science* **275,** 1649–1651.
28. Puri, P. L., Avantaggiati, M. L., Burgio, V. L., Chirillo, P., Collepardo, D., Natoli, G., Balsano, C., and Levrero, M. (1995) *J. Biol. Chem.* **270,** 22129–22134.
29. Lo, Y. Y., and Cruz, T. F. (1995) *J. Biol. Chem.* **270,**11727–11730.
30. Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B., and Rhee, S. G. (1997) *J. Biol. Chem.* **272,** 217–221.
31. Chandra, S., Martin, G. B., and Low, P. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 13393–13397.
32. Ohta, Y., Torii, H., Okada, H., Majima, Y., and Ishiguro, I. (1996) *Curr. Eye Res.* **15,** 1–7.
33. Takata, M., Sabe, H., Hata, A., Inazu, T., Homma, Y., Nukada, T., Yamamura, H., and Kurosaki, T. (1994) *EMBO J.* **13,** 1341–1349.
34. Cass, A., and Dalmark M. (1973) *Nature* **244,** 47–49.
35. Kurosaki, T., Takata, M., Yamanashi, Y., Inazu, T., Taniguchi, T., Yamamoto, T., and Yamamura, H. (1994) *J. Exp. Med.* **179,** 1725–1729.
36. Qin, S., Inazu, T., and Yamamura, H. (1995) *Biochem. J.* **308,** 347–352.
37. Parker, J. C. (1993) *Am. J. Physiol.* **265,** C1191–C1200.
38. Heldin, C. H. (1995) *Cell* **80,** 213–223.
39. Sachsenmaier, C., Radler-Pohl, A., Zinck, R., Nordheim, A., Herrlich, P., and Rahmsdorf, H. J. (1994) *Cell* **78,** 963–972.
40. Cheng, A. M., Rowley, B., Pao, W., Hayday, A., Bolen, J. B., and Pawson, T. (1995) *Nature* **378,** 303–306.
41. Taniguchi, T., Kitagawa, H., Yasue, S., Yanagi, S., Sakai, K., Asahi, M., Ohta, S., Takeuchi, F., Nakamura, S., and Yamamura, H. (1993) *J. Biol. Chem.* **268,** 2277–2279.
42. Turner, M., Mee, J., Costello, P. S., Williams, O., Price, A. A., Duddy, L. P., Furlong, M. T., Geahlen, R. L., and Tybulewicz, V. L. (1995) *Nature* **378,** 298–302.
43. Nakashima, I., Pu, M. Y., Nishizaki, A., Rosil, I., Ma, L., Katano, Y., Ohkusu, K., Rahman, S. M., Isobe, K., Hamaguchi, M., and Saga, K. (1994) *J. Immunol.* **152,** 1064–1071.
44. Guyton, K. Z., Liu, Y., Gorospe, M., Xu, Q., and Holbrook, N. J. (1996) *J. Biol. Chem.* **271,** 4138–4142.
45. Takeuchi, F., Taniguchi, T., Maeda, H., Fujii, C., Takeuchi, N., and Yamamura, H. (1993) *Biochem. Biophys. Res. Commun.* **194,** 91–96.
46. Yamada, T., Taniguchi, T., Nagai, K., Saitoh, H., and Yamamura, H. (1991) *Biochem. Biophys. Res. Commun.* **180,** 1325–1329.