Heat Stress Induces a Glycosylation of Membrane Sterol in Myxamoeba of a True Slime Mold, Physarum polycephalum*

(Received for publication, July 9, 1996, and in revised form, September 17, 1996)

Kimiko Murakami-Murofushi‡§, Keiko Nishikawa‡, Emi Hirakawa‡, and Hiromu Murofushi¶

From the ‡Department of Biology, Faculty of Science, Ochanomizu University, Tokyo 112, Japan and §Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Tokyo 113, Japan

To know the very early events occurring after heat shock, the changes of membrane lipids were examined. Heat stress induced the production of a certain glycolipid in the myxamoeba of Physarum polycephalum in a few minutes. The purified glycolipid was determined to be a poriferasterol monoglucoside by structural studies that were previously reported to be expressed during the differentiation of Physarum cells from haploid myxamoebae to diploid plasmodia (Murakami-Murofushi, K., Nakamura, K., Ohta, J., Suzuki, M., Suzuki, A., Murofushi, H., and Yokota, T. (1987) Biochim. Biophys. Acta 992, 412–415) was also expressed rapidly after heat shock. Thus, the activation of sterol glucosyltransferase and the production of sterol-glucoside were considered to be important events that were involved in the signal transduction system to induce some succeeding heat-shock responses, such as the synthesis of heat-shock proteins.

In response to environmental stresses, living organisms acquire the capability of recognizing such stresses and adapting themselves to various types of stress during evolution. They induce some proteins, so-called stress proteins or heat-shock proteins, to protect themselves from conditions unfavorable for their survival.

Under stress conditions such as heat shock, starvation, high salt, and high osmotic pressure, haploid myxamoebae of a true slime mold, Physarum polycephalum, retracted their pseudopodia and changed shape into a disk-like form, then they constructed cell walls to form their dormant form, microcysts. These morphological changes were associated with changes in the intracellular distribution of actin filaments. Synthesis of a novel stress protein, p66, was induced within 15 min after heat shock. Because this protein coprecipitated with polymerized actin in vitro and colocalized with short bundles of actin filaments in vivo, it may have participated in the change of actin distribution associated with heat-inducible microcyst formation. However, p66 was not induced when diploid plasmodia of Physarum were exposed to heat shock, so this protein is considered to be specifically expressed during microcyst formation in the haploid stage of Physarum (3). The structure of the p66 gene, the biological functions of p66, and the regulation of actin-p66 binding are now being investigated in our laboratory.

In many organisms, the induction and properties of heat-shock proteins have been investigated in detail (4, 5), and many investigators are now working on this subject. However, very early events after heat shock have not yet been clarified. Because the plasma membrane may receive a heat shock first and then the signal may be transduced into the cell, we studied the change of membrane lipids after heat shock and demonstrated a rapid induction of a certain glycolipid and its synthesizing enzyme in myxamoeba of P. polycephalum.

EXPERIMENTAL PROCEDURES

Organisms—Myxamoeba of a true slime mold, P. polycephalum, were grown on a lawn of bacteria, Aerobacter aerogenes, in a nutrient agar medium in the dark at 24 °C (6). For heat shock, they were incubated at 40 °C.

Chemicals—Sephadex A-25 was obtained from Pharmacia Fine Chemicals. Silia gel 60 thin layer chromatography (TLC) plates and high-performance TLC plates of silica gel 60 were from Merck, and 3% gas-liquid chromatography.

EXPERIMENTAL PROCEDURES

Gas-Liquid Chromatography (GLC)—For the detection of components of a purified glycolipid, homogeneous substance was methanolized, and the resultant methyl glycoside and sterol were trimethylsilylated and analyzed by GLC on a column of 3% OV-101 as described (1).

Fast Atom Bombardment Mass Spectrometry—Fast atom bombardment mass spectrometry for an intact glycolipid was carried out on a JEOL DX-303 mass spectrometer under the described conditions (1).

Enzymatic Hydrolysis—The glycolipid was dissolved in 50 mM sodium citrate, pH 5.0, and treated with α- or β-glucosidase as described (1).

Assay of UDP-glucose:Poriferasterol Glucosyltransferase—The assay of this transferase was performed essentially by the procedure of

* This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and from Salt Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 81-3-5978-5362; Fax: 81-3-5978-5362.
RESULTS

Expression of a Glycolipid in the Heat-shocked Myxamoebae—When myxamoebae were incubated at 40 °C, the induction of a certain glycolipid was prominent soon after the temperature shift. *Physarum* myxamoebae were heat-treated at 40 °C for various periods, and crude lipid fractions that were extracted from heat-shocked cells were analyzed by TLC. A certain glycolipid, designated GL-X (Fig. 1), appeared just after the temperature shift and increased in amount for about 10 min. A content of this substance was maintained constantly for at least 60 min. The expression of this substance was followed by the induction of stress protein p66 and some other heat-shock proteins and microcyst formation as shown in Fig. 2. A slight change of glycolipid C (Fig. 1) was also observed, but it was already present in significant quantities before the temperature shift. The chemical nature of glycolipids A–D and the meaning of the slight change of glycolipid C have not yet been studied.

Purification and Structural Studies of a GL-X—GL-X was purified as described under "Experimental Procedures," and the purity of this substance was analyzed by TLC in different solvent systems (I and II) and with some different color-developing reagents for the detection of some biochemical compounds. GL-X showed a single spot in each solvent system used and visualized with orcinol-H$_2$SO$_4$ and ferric chloride solution. No other reagents tested reacted with GL-X. Fig. 3 shows the two-dimensional TLC analysis of GL-X visualized with orcinol reagent (A) and with ferric chloride reagent (B). Hence, GL-X is shown to be composed of hexose and sterol.

The purified GL-X was subjected to a negative fast atom bombardment mass spectrometry. The [M-1]$^-$ ion at m/e 573 was obtained, then the molecular weight of GL-X was determined to be 574 (Fig. 4). This molecular weight is identical with that of poriferasterol monoglucoiside that was isolated in *Physarum* plasmodia (1).

GL-X was methanolized, and trimethylsilyl derivatives of methyl glycoside and sterol were analyzed by GLC as shown in Figs. 5 and 6. From these results, the sugar moiety and sterol moiety of GL-X were determined as glucose and poriferasterol, respectively. No other components were detected by GLC analysis under some different conditions with some different columns.

Because GL-X was hydrolyzed with β-glucosidase but not α-glucosidase (data not shown), the linkage of glucose β-poriferasterol was suggested. From a colorimetric determination of glucose and sterol, a molar ratio of 1.0:1.1 was obtained.

Induction of UDP-glucose:Poriferasterol Glucosyltransferase Activity in Heat-shocked Myxamoebae—Endogenous UDP-glucose:poriferasterol glucosyltransferase activities were assayed in the homogenates of haploid myxamoebae before and after temperature shift from 24 °C to 40 °C. The enzyme activity was not detected in the homogenate before heat shock, but an apparent activation of UDP-glucose:poriferasterol glucosyltransferase was observed after heat shock as shown in Fig. 8.
DISCUSSION

In this report, we showed a rapid expression of poriferasterol monoglucoside by heat shock at 40°C in the haploid myxamoebae of *P. polycephalum*. The enzyme UDP-glucose:poriferasterol glucosyltransferase was also activated rapidly after heat shock to form the above-mentioned glycolipid. From these findings, rapid production of steryl glucoside might be involved in the very early process of stress response of *Physarum* myxamoebae.

Previously, we reported the expression of this substance during the differentiation of *Physarum* cells from haploid myxamoebae to diploid plasmodia (1), and an expression of UDP-glucose:poriferasterol glucosyltransferase activity associated with the differentiation was also demonstrated (2). It takes about 1 week after mating for myxamoebae to differentiate into plasmodia, but the steryl glucoside and its synthesizing enzyme appeared at an early stage of differentiation (1 day after the mating of haploid cells).

The steryl glucoside and its 6'-O-acyl derivatives are known as common constituents of higher plants (13, 14), and their functions are considered to be metabolically active components of plant membrane structure (15), intercellular transporters of sterols (16), or glucose carriers through cell membranes (17, 18). In *Physarum*, plasmodia are capable of growth in liquid or on agar media, but myxamoebae, except for the rare mutant strain *Colonia* (19), can be cultured only on bacterial lawns. Myxamoebae may not be able to utilize glucose and other small molecules, but plasmodia are capable of utilizing them as nutrients. Poriferasterol monoglucoside may have some active functions in membranes showing such interesting properties.

A matingless mutant, *Colonia* strain, differentiates from myxamoebae into plasmodia without any changes of nuclear DNA. When the cultivating temperature is reduced from 30°C to 25°C, the myxamoebae differentiate into plasmodia with-
out conjugation, and then haploid, not diploid, plasmodia are formed. We showed that the cells of this mutant strain contained poriferasterol monoglucoside and its synthesizing enzyme in both the myxamoeboid and plasmodial stages. We also demonstrated that Colonia cells showed a slower rate of growth than that of wild-type ones on a lawn of bacteria, but they could survive and increase their cell number even in culture medium (20). This indicates that the Colonia myxamoebae can uptake some small substances from the nutrient media as their energy source. Then the uptake of glucose and amino acids into myxamoebae was measured. The results clearly showed a much higher uptake of D-glucose into Colonia cells than into wild-type cells. The uptake ability of amino acids into wild-type and mutant myxoamoebae was examined, and no difference between them was observed. Because almost no differences were observed in the composition of other membrane components between wild-type and Colonia myxamoebae, these results strongly suggest the involvement of poriferasterol monoglucoside in the active transport of D-glucose.

Then we discussed that this substance may assist or regulate the action of glucose transporter protein in plasma membrane (20).

This substance may also be considered to act as an accelerator on the fusion of plasma membrane because the fusion of plasma membranes of mutant myxoamoebae occurred when the temperature was reduced, but wild-type myxoamoebae could fuse only in the case of conjugation of the cells of different mating types.

The biological significance of heat induction of steryl glycoside has not yet been clarified, but this substance may have some important role(s) in the process of heat-induced differentiation. It may act at an early step in a signal transduction system to trigger stress-induced differentiation; for example, it may regulate the heat-receptor on the membrane or assist a movement of active molecules in the membrane to induce successive heat responses. Another possibility is that this substance by itself may act as a mediator in a signal transduction system of heat stress. Additional investigations are necessary to clarify the biological significance of a glycosylation of membrane sterol in heat response and cell differentiation.

Recently, we also found a heat-induced expression of steryl glycoside in human cultured cells, and the purification and characterization of this substance are now underway. Hence, this phenomenon is not specified in Physarum cells and might have some important role(s) in all organisms.

Acknowledgments—We are indebted to Dr. Ichiro Yahara of the Tokyo Metropolitan Institute of Medical Sciences and Dr. Kazuhiro Nagata of Kyoto University for their encouragement and valuable discussion on this study.

REFERENCES

1. Murakami-Murofushi, K., Nakamura, K., Ohta, J., Suzuki, M., Suzuki, A., Murofushi, H., and Yokota, T. (1987) J. Biol. Chem. 262, 16719–16723
2. Murakami-Murofushi, K., and Ohta, J. (1986) Biochim. Biophys. Acts 992, 412–415
3. Shimada, Y., Kasakura, T., Yokota, M., Miyata, Y., Murofushi, H., Sakai, H., Yahara, I., and Murakami-Murofushi, K. (1992) Cell Struct. Funct. 17, 301–309
4. Schlesinger, M. J., Ashburner, M., and Tissieres, A. (eds) (1982) Heat Shock from Bacteria to Man, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
5. Lindquist, S., and Craig, E. A. (1988) Annu. Rev. Genet. 22, 631–677
6. Murakami-Murofushi, K., Hiratsuka, A., and Ohta, J. (1984) Cell Struct. Funct. 9, 311–315
7. Murakami-Murofushi, K., Nakamura, K., Ohta, J., and Yokota, T. (1987) Cell Struct. Funct. 12, 519–524
8. Mimura, K., Murakami-Murofushi, K., and Ohta, J. (1991) Nat. Sci. Rep. Ochanomizu Univ. 42, 49–56
9. Wojciechowski, Z. A., Zinowski, J., and Tyski, S. (1977) Phytochemistry 16, 911–914
10. Wojciechowski, Z. A., Zinowski, J., Zinowski, J. G., and Lyznik, A. (1979) Biochim. Biophys. Acts 570, 363–370
11. Radin, N. S., Lavin, F. B., and Brown, J. R. (1955) J. Biol. Chem. 217, 789–796
12. Momose, T., Ueda, Y., Yamamoto, K., Masumura, T., and Ohta, K. (1963) Anal. Chem. 35, 1751–1753
13. Lepage, M. (1964) J. Lipid Res. 5, 587–592
14. Bush, P. B., and Grunwald, C. (1972) Plant Physiol. 50, 69–72
15. Grunwald, C. (1971) Plant Physiol. 48, 655–665
16. Evans, F. J. (1972) J. Pharm. Pharmacol. 24, 645–650
17. Smith, P. F. (1969) Lipids 4, 331–336
18. Wojciechowski, Z. A., Zinowski, J., and Zielenska, M., (1976) Phytochemistry 15, 1681–1683
19. McCullough, C. H. R., Dee, J., and Foxon, J. L. (1978) J. Gen. Microbiol. 106, 297–306
20. Murakami-Murofushi, K., Kamiya, Y., Mimura, K., and Ohta, J. (1990) Proc. Jpn. Acad. 66, 197–202
Heat Stress Induces a Glycosylation of Membrane Sterol in Myxoamoebae of a True Slime Mold, *Physarum polycephalum*

Kimiko Murakami-Murofushi, Keiko Nishikawa, Emi Hirakawa and Hiromu Murofushi

*J. Biol. Chem.* 1997, 272:486-489.  
doi: 10.1074/jbc.272.1.486

Access the most updated version of this article at http://www.jbc.org/content/272/1/486

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 19 references, 5 of which can be accessed free at http://www.jbc.org/content/272/1/486.full.html#ref-list-1