J. Biochem. 119, 354-359 (1996)

Transmethylation Reaction Is Essential for *Sarcophaga* Lectin Gene Activation[1]

Hiromi Sugiyama and Shunji Natori[2]

Faculty of Pharmaceutical Science, The University of Tokyo, Bunkyo-ku, Tokyo 113

Received for publication, October 18, 1995

Incubation of *Sarcophaga* fat bodies in buffered insect saline resulted in simultaneous activation of multiple defense protein genes [Sugiyama, H. and Natori, S. (1991) *Eur. J. Biochem.* 200, 495-500]. In this study, we demonstrated that the addition of both adenosine and homocysteine thiolactone to this medium abolished activation of these defense protein genes. As *S*-adenosyl-homocysteine, a potent inhibitor of the transmethylation reaction, is produced in the fat body under these conditions, methylation of a certain fat body protein seems to be a prerequisite for the selective activation of insect defense protein genes. Activation of the HSP70 gene was not interfered with *S*-adenosyl-homocysteine.

Key words: defense protein genes, NF-$\varkappa$B, transmethylation, insect, *Sarcophaga* lectin.

Insects are known to respond promptly to bacterial infection and/or body injury by producing various humoral defense proteins (*1*). These are diverse sets of broad-spectrum antimicrobial proteins and lectins that are synthesized by the fat body and/or hemocytes (*2-13*). Many of these defense protein genes share a common upstream motif similar to the binding site for NF-$\varkappa$B (*14, 15*) a mammalian member of the *Rel* family of transcription factors (*16, 17*). Sun and Faye purified a factor with NF-$\varkappa$B-like properties (CIF) from *Hyalophora cecropia* pupae (*18*). Recently, we purified a protein with a molecular mass of 59 kDa that specifically binds to NF-$\varkappa$B-binding motifs of the defense protein genes of *Sarcophaga peregrina* (*19*). This protein is likely to be a NF-$\varkappa$B-like transcription factor.

In a previous study, we demonstrated that the genes for various *Sarcophaga* defense proteins, namely *Sarcophaga* lectin, and sarcotoxins IA and IIA, were activated when the larval fat body was simply incubated in phosphate-buffered insect saline (BIS) *in vitro* and the addition of 2-mercaptoethanol (2-ME) abolished activation of these genes (*20*). Subsequently, we found that the glutathione (GSH) content of the fat body decreased significantly under these conditions (*21*). As the cellular GSH content is known to be crucial for mammalian NF-$\varkappa$B activation (*22*), we speculated that a transient decrease in the GSH level in the fat body is essential for NF-$\varkappa$B-like transcription factor activation.

In this paper, we report a novel aspect of *Sarcophaga* defense protein gene activation. We demonstrated that an intracellular transmethylation reaction, possibly methylation of a certain protein, is a prerequisite for the specific activation of defense protein genes in the fat body. We also suggest that selective inhibition of the activation of these defense protein genes in the fat body by 2-ME is partly due to inhibition of the transmethylation reaction by 2-ME.

MATERIALS AND METHODS

Animals—Flesh flies, *Sarcophaga peregrina*, were kept at 27°C with dried milk, sugar cubes, and fresh water. The larvae were reared on pork liver and when they reached the third instar, and crawled upward, they were collected, washed well with water, and kept in a plastic container with a small amount of water. Larvae could be used for experiments for up to 3 days after leaving their food.

Fat-Body Culture in BIS—This was performed essentially as described previously (*20*). Briefly, the fat body was excised from each third instar larva under a binocular microscope and rinsed well with ice-cold insect saline (130 mM NaCl, 5 mM KCl, and 1 mM $CaCl_2$). Each fat body was placed in 300 μl BIS (10 mM NaH_2PO_4/Na_2HPO_4, pH 6.2, 2 mM $NaHCO_3$, 1 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, and 120 mM NaCl). When required, 2-ME, dithiothreitol (DTT), cysteine, adenosine (Ado), and L-homocysteine thiolactone (HcyTL), at the indicated final concentrations, were added to the culture medium. The tissues were incubated in 24-well flat-bottomed tissue culture plates at 27°C for the indicated times in a humidified air atmosphere, after which each fat body was removed from the medium, frozen immediately on dry ice, and stored at −80°C until analyzed.

Northern Blot Hybridization—The RNA was extracted from each fat body, as described elsewhere (*20*), and subjected to electrophoresis using a horizontal slab of 1.2% agarose gel containing 2.2 M formaldehyde in 20 mM 3-[*N*-morpholino]propanesulfonic acid, 5 mM sodium ace-

[1] This work was supported by a Grant-in-Aid for Specifically Promoted Research from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid MBP (Bio Media Program) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

[2] To whom correspondence should be addressed. Fax: +81-3-5684-2973, Phone: +81-3-3812-2111 (Ext. 4820)

Abbreviations: BIS, buffered insect saline; 2-ME, 2-mercaptoethanol; GSH, glutathione; DTT, dithiothreitol; Ado, adenosine; HcyTL, L-homocysteine thiolactone; AdoHcy, *S*-adenocyl-L-homocysteine; HSP, heat shock protein; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography; RT, reverse transcription; PCR, polymerase chain reaction; CHX, cycloheximide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

tate, and 1 mM EDTA, pH 7.0 (buffer A). Before application to the gel, the RNA was heated at 60°C for 7 min in buffer A containing 50% formamide and 2.2 M formaldehyde. After electrophoresis, the RNA was transferred from the gel to nitrocellulose filter paper, as described by Thomas (*23*), and hybridized with DNA probes labeled with [α-^{32}P]dCTP. The hybridization mixture consisted of 50% formamide, 5×NaCl/Cit [NaCl/Cit: 0.15 M sodium chloride and 15 mM trisodium citrate], 1×Denhardt's solution [Denhardt's solution: 0.02% each of bovine serum albumin, poly(vinylpyrrolidone) 40, and Ficoll 400], 50 mM sodium phosphate, pH 6.5, 100 μg/ml denatured and sonicated salmon sperm DNA and 0.1% SDS.

Before hybridization, the filter was incubated without probe DNA under the above conditions, except that 1% glycine was added. Hybridization with a radiolabeled probe was carried out at 42°C for 12-16 h, the filters were washed with 2×NaCl/Cit containing 0.1% SDS for 15 min at room temperature, then with 0.1×NaCl/Cit containing 0.1% SDS for 15 min at 47°C, and finally autoradiographed at −80°C.

A cDNA clone for *Sarcophaga* lectin, pLE10 (*24*), was used throughout to detect *Sarcophaga* lectin mRNA and pTΠ20 (*25*), pS25 (*26*), and a fragment of *Sarcophaga* HSP70 cDNA were used as controls to detect other fat body protein mRNAs.

Cloning of a Fragment of Sarcophaga HSP70 cDNA—A fragment of *Sarcophaga* HSP70 cDNA was amplified by the RT-PCR and cloned. Briefly, each fat body was incubated in BIS containing 1 mM Ado and 1 mM HcyTL at 25°C for 1 h. Then the temperature was increased to 37°C, incubation was continued for 1 h and the total RNA was extracted and used as a template for the RT-PCR. Sense and antisense primers, based on the sequence of *Drosophila* HSP70 were synthesized (*27*). The primers used were 5′-TCG ATG GTG CTG AC(G/C) AAG ATG AAG GA-3′ and 5′-CGT GCT AGA GGA GAG TGT GCG CTT-3′ that correspond to amino acids 118-126 and 269-276, respectively. The PCR product (477 bp) was labeled with [^{32}P]-dCTP and used as a probe for Northern blotting hybridization to detect HSP70 mRNA.

Detection of S-Adenosyl-L-Homocysteine—This was performed essentially as reported by Gordon *et al.* (*28*). Each fat body was homogenized in 200 μl of 100 mM methanesulfonic acid and the homogenate was centrifuged at 8,000×*g* for 15 min. The resulting supernatant (50 μl) was subjected to HPLC using a Radial-Pak SCX cartridge (Waters). *S*-Adenosyl-L-homocysteine (AdoHcy) was eluted from the column with a linear gradient of buffer A (1 mM NH_4COOH/HCOOH, pH 4.0) and buffer B [0.2 M NH_4-COOH/HCOOH containing 0.8 M $(NH_4)_2SO_4$, pH 4.0]. Elution of the material was monitored by measuring A_{254}.

Assay of Protein Carboxyl Methylation in the Fat Body—Carboxyl methylation of the fat-body protein was measured using a vapor-phase equilibrium procedure (*29*). Each fat body was cultured in 300 μl BIS containing [methyl-^{3}H]-Met for 30 min at 27°C, then washed with BIS and homogenized in 500 μl extraction buffer (10 mM Tris/HCl, pH 7.2, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA). Then the homogenate was centrifuged at 11,000×*g* for 15 min, the methylated protein in the resulting supernatant was precipitated with 5% trichloroacetic acid, treated with 1 N NaOH for 40 h at 37°C, the resulting [^{3}H]methanol released from the protein was trapped in a scintillation fluid and its radioactivity was measured.

Analysis of Methylated Protein in the Fat Body—Each fat body was cultured in 300 μl BIS containing 30 μCi [^{3}H]Met and 100 μM cycloheximide (CHX) for 30 min at 27°C. When required, 2-ME, Ado, and HcyTL, at indicated final concentrations, were added to the culture medium. Then the fat body was washed well with BIS and homogenized in 500 μl extraction buffer to prepare fat body extract. The resulting extract was subjected to 15% SDS-polyacrylamide gel electrophoresis followed by fluorography.

RESULTS

Suppression of Sarcophaga Lectin Gene Expression by Inhibition of the Transmethylation Reaction in the Fat Body—As reported previously, 2-ME suppressed *Sarcophaga* lectin gene activation in the *Sarcophaga* fat body *in vitro* (*20*). However, we found that DTT and cysteine did not affect this reaction (Fig. 1). It is generally known that 2-ME is detoxicated by thiol *S*-methyltransferase in the cell (*30*), whereas polar thiol compounds, such as DTT and cysteine, are not methylated by this enzyme (*31, 32*). Therefore, we examined the behavior of 2-ME by monitoring the sulfhydryl group levels in the culture medium with time and found that 2-ME disappeared rapidly (Fig. 2), while without fat body, only a slight decrease of 2-ME by auto-oxidation was observed. These results suggest that 2-ME entered the fat body cells (adipocytes) and was detoxicated *in situ* by thiol *S*-methyltransferase. As expression of the *Sarcophaga* lectin gene was suppressed only in the presence of 2-ME, we concluded that methylation of 2-ME is closely related to *Sarcophaga* lectin gene expression.

In order to examine the relationship between the transmethylation reaction and *Sarcophaga* lectin gene expression in this system, we treated fat bodies with both Ado and HcyTL to induce AdoHcy production in the fat body (*33, 34*). AdoHcy is a potent inhibitor of methyltransferase (*35*), but as cells are not permeable to it, so a method of producing AdoHcy *in vivo* from Ado and HcyTL has been established (*33, 34*). As shown in Fig. 3A, the *Sarcophaga* lectin gene was repressed completely in the presence of both Ado and HcyTL, but neither component alone inter-

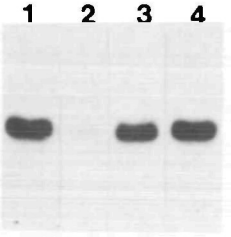

Fig. 1. **Effects of 2-ME and DTT on *Sarcophaga* lectin gene activation.** Fat bodies were cultured in BIS for 2 h at 27°C in the presence of 0.5 mM 2-ME or 10 mM DTT or 10 mM cysteine, then RNA was extracted from the fat bodies and *Sarcophaga* lectin gene expression was examined by Northern blot hybridization. Lane 1, fat body cultured in BIS alone (control); lane 2, fat body cultured with 2-ME; lane 3, fat body cultured with DTT; lane 4, fat body cultured with cysteine.

fered with its expression. In the presence of both Ado and HcyTL, sarcotoxin IIA gene expression was also suppressed (Fig. 3B), but the 25-kDa protein (a constitutive protein of the fat body) gene expression was not affected (Fig. 3C).

These results suggest strongly that defense protein gene expression is inhibited specifically when the transmethylation reaction is inhibited by AdoHcy. In order to confirm this, we examined the effect of AdoHcy on the expression of the *Sarcophaga* HSP70 gene, which is also inducible. When third instar larvae were heated at 37, 39, and 41°C for 1 h, the *Sarcophaga* HSP70 gene in the fat body was activated significantly (Fig. 4A), whereas no appreciable activation of the *Sarcophaga* lectin gene was detected (Fig. 4B). These results indicate that the HPS70 gene was activated selectively when *Sarcophaga* larvae were heated over 37°C.

Next, in order to examine the effect of AdoHcy on HSP70 gene expression, we first induced AdoHcy production in the fat body at 25°C and then increased the culture medium temperature to 37°C to activate the HSP70 gene. As shown in Fig. 5, the HSP70 gene was activated at 37°C even in the presence of AdoHcy, whereas under the same conditions, the *Sarcophaga* lectin gene was not activated at all. These results demonstrate clearly that AdoHcy selectively inhibited activation of the *Sarcophaga* lectin gene, but not that of the HSP70 gene.

Evidence for the Production of AdoHcy and Suppression of Protein Transmethylation in the Fat Body by Treating It with Ado and HcyTL In Vitro—It became evident that treating the fat body with Ado and HcyTL selectively

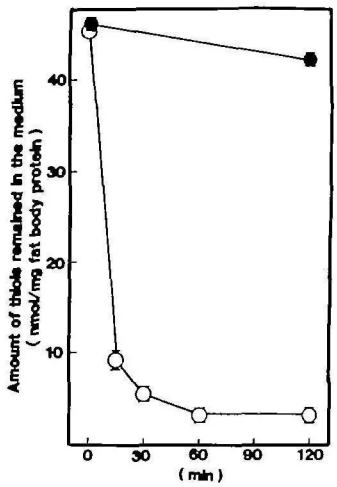

Fig 2. **Reduction of the 2-ME concentration in the fat-body culture medium.** Fat bodies were cultured in BIS containing 0.5 mM 2-ME Medium samples were taken after various incubation times and the amount of thiols remaining in the medium was determined as follows, 50 μl of the culture medium was mixed with 150 μl of 0.5 mM DTNB solution dissolved in 0 15 M phosphate buffer (pH 7 5) containing 6 3 mM EDTA and measured A_{412} The values were normalized on the basis of the fat-body protein level. The points and bars represent the means and standard errors of the values of 6 measurements. Open circles indicate the values with fat body, and closed circles, without fat body, respectively

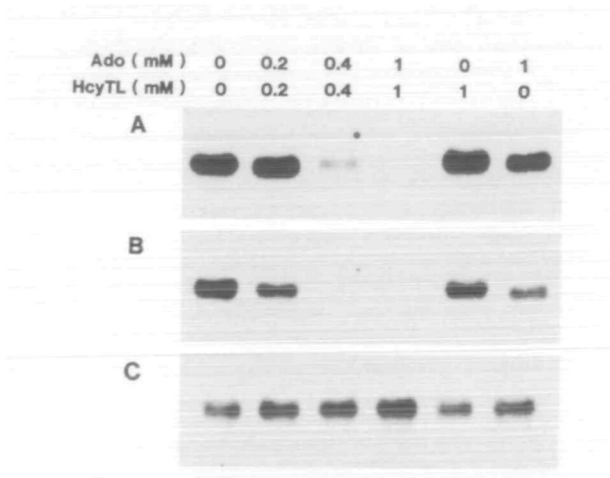

Fig. 3 **Effect of AdoHcy produced *in vivo* on defense protein gene expression.** Fat bodies were incubated in BIS in the presence of various concentrations of Ado and HcyTL for 2 h at 27°C, the RNA was extracted from each fat body and expression of the *Sarcophaga* lectin (A), sarcotoxin IIA (B), and the 25-kDa protein (C) genes were examined by Northern blot hybridization.

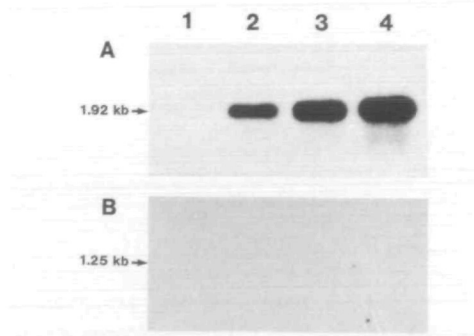

Fig. 4 ***Sarcophaga* HSP70 gene activation.** Third instar larvae were heated in a water bath for 1 h, the fat bodies were harvested, the RNA extracted and analyzed by Northern blot hybridization using the 477-bp fragment of *Sarcophaga* HSP70 cDNA (A) or *Sarcophaga* lectin cDNA (B) Lane 1, control larvae (not heat treated), lane 2, larvae heated at 37°C; lane 3, larvae heated at 39°C; lane 4, larvae heated at 41°C Arrows indicate HSP70 mRNA (1.92 kb) and *Sarcophaga* lectin mRNA (1 25 kb), respectively

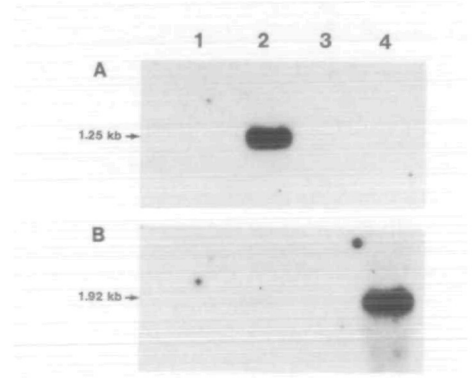

Fig. 5 **Effect of AdoHcy on HSP70 gene activation.** Fat bodies were treated first with 1 mM each Ado and HcyTL for 2 h at 25°C in BIS, then the temperature was increased to 37°C and incubation was continued for 1 h, the RNA was extracted from each fat body and *Sarcophaga* lectin (A) and HSP70 gene (B) expression was examined by Northern blot hybridization The fat bodies from which RNAs were extracted were treated as follows: lane 1, control (no incubation); lane 2, incubated in BIS at 25°C for 3 h; lane 3, incubated in BIS containing Ado and HcyTL for 3 h at 25°C; lane 4, incubated in BIS containing Ado and HcyTL for 2 h at 25°C and then at 37°C for 1 h.

suppressed *Sarcophaga* lectin gene activation Therefore, we investigated whether AdoHcy was actually produced in the fat body under these conditions. Fat bodies treated with Ado and HcyTL were homogenized, and the resulting extracts were subjected to HPLC to detect AdoHcy. As shown in Fig. 6, a clear AdoHcy peak was detected only in the extracts of fat bodies treated with Ado and HcyTL, indicating that AdoHcy was synthesized by and accumulated in the fat body.

Next, we examined the efficiency of protein methylation under these conditions. Fat bodies were labeled with [methyl-^{3}H]Met in the presence of Ado and HcyTL and the radioactive methyl groups that transferred to carboxyl groups of proteins were trapped as [^{3}H]methanol (*29*), the radioactivity of which was measured. As summarized in Table I, alkaline-labile methylester formation of fat-body protein (carboxyl methylation) (*36*) was inhibited by 75% in the presence of 1 mM both Ado and HcyTL. We assumed this inhibition was due to AdoHcy produced under these conditions. We also examined the effect of 2-ME on this methylation reaction and found that it was inhibited by 55% in the presence of 1 mM 2-ME. These results suggest that both AdoHcy and 2-ME (*37*) inhibit the protein carboxyl methylation reaction in the fat body, which results in suppression of *Sarcophaga* lectin gene expression. This transmethylation reaction was not required for the expression of the inducible HSP70 gene, suggesting that it is a specific reaction needed for expression of the genes regulated by *Rel* family transcription factors.

Analysis of Methylated Protein in the Fat Body—To examine methylated protein in the fat body, we cultured fat

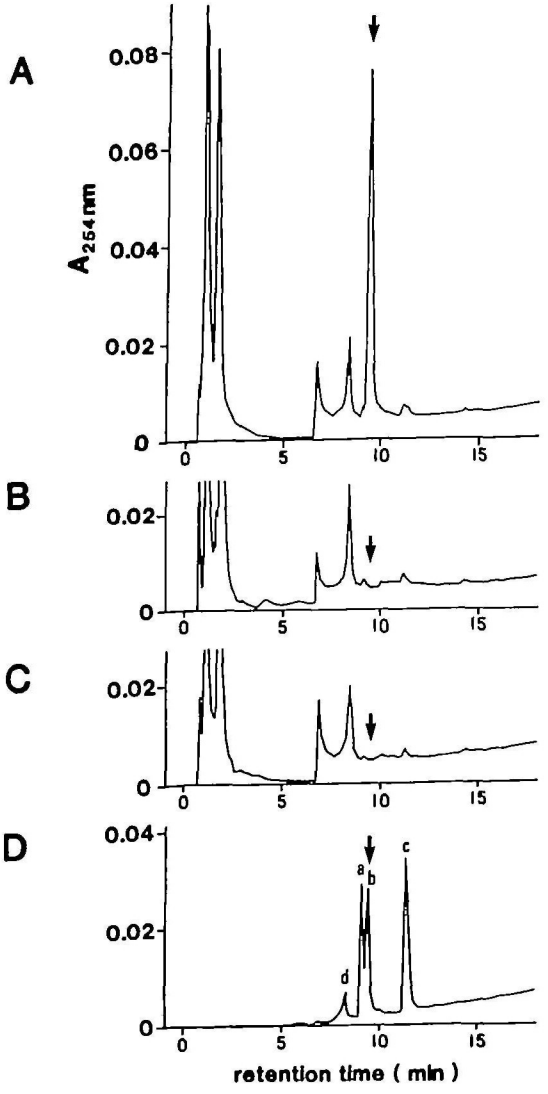

Fig 6. **Accumulation of AdoHcy in fat bodies treated with Ado and HcyTL.** Fat bodies were incubated in BIS containing 1 mM each Ado and HcyTL for 30 min at 27°C, then homogenized, and the amount of AdoHcy in each homogenate was analyzed by HPLC. (A) Fat body incubated in the presence of Ado and HcyTL. (B) Fat body incubated in the absence of Ado and HcyTL. (C) Unincubated fat body. (D) Elution profile of standard compounds. a, Ado (1 nmol), b, AdoHcy (1 nmol), c, HcyTL (10 nmol); d, methanesulfonic acid background (2 μmol) The arrows indicate AdoHcy peaks

TABLE I **Effects of Ado+HcyTL and 2-ME on protein carboxyl methylation.** Fat bodies were labeled with [methyl-^{3}H]Met in the presence of both Ado and HcyTL or 2-ME for 30 min, and the radioactivity of the methyl groups transferred to the protein was measured as described previously Protein carboxyl methylation was calculated as the volatile radioactivity relative to the total radioactivity, and expressed as a percentage The means of duplicate measurements with deviations are shown.

Treatment	Concentration of reagent (mM)	Protein carboxyl methylation (%)	Reduction of carboxyl methylation (%)
None	0	5 1±1 4	0
Ado+HcyTL	1 0	1 3±0.1	75
2-ME	1 0	2 3±0 6	55

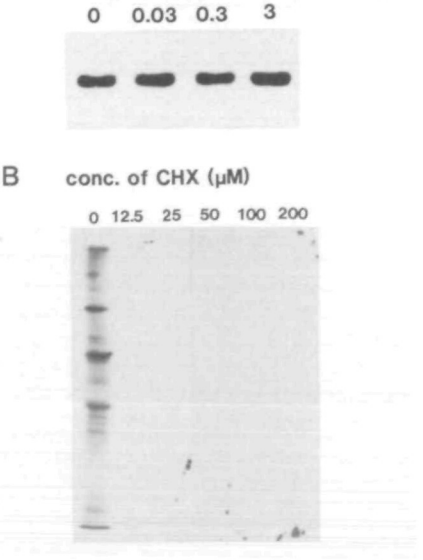

Fig 7 **Effect of CHX on *Sarcophaga* lectin gene expression and protein synthesis.** (A) Fat bodies were cultured in BIS containing indicated concentrations of CHX for 2 h at 27°C. Then RNA was extracted from the fat bodies and *Sarcophaga* lectin gene expression was examined by Northern blot hybridization. (B) Fat bodies were incubated in BIS containing indicated concentration of CHX and 10 μCi [^{35}S]Met for 30 min at 27°C Labeled protein was analyzed by SDS-polyacrylamide gel electrophoresis followed by fluorography. Each lane contained 60 μg protein.

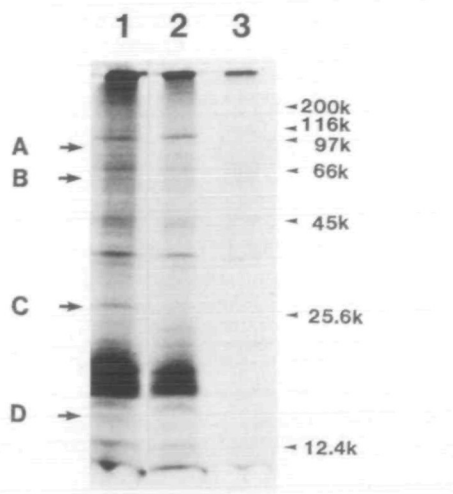

Fig 8 **Fluorography of methylated protein.** Fat bodies were cultured in BIS containing 100 μM CHX and 30 μCi [methyl-^{3}H]Met in the presence of 1 mM 2-ME or 1 mM each of Ado and HcyTL for 30 min at 27°C Fat body extract was prepared and analyzed by SDS-polyacrylamide gel electrophoresis followed by fluorography Each lane contained 50 μg protein Arrows A to D indicate proteins of which methylation was inhibited by 2-ME Lane 1, fat body cultured in BIS (control) alone, lane 2, fat body cultured with 2-ME, lane 3, fat body cultured with Ado and HcyTL.

body in BIS in the presence of 100 μM CHX and labeled proteins with [^{3}H]Met. Under these conditions, no appreciable protein synthesis was detected, but activation of the *Sarcophaga* lectin gene was not affected by CHX (Fig. 7). Therefore, proteins are labeled only by transmethylation under these conditions. As is evident from Fig. 8, various proteins were methylated and this transmethylation reaction was inhibited almost completely by AdoHcy, but partly by 2-ME. As pointed out by arrows A to D, methylation of some proteins seemed to be inhibited preferentially in the presence of 2-ME. Possibly, these proteins are related to the activation of the *Sarcophaga* lectin gene.

DISCUSSION

In a previous study, we demonstrated that when *Sarcophaga* larval fat bodies are simply incubated in a phosphate-buffered insect saline, various defense protein genes are activated selectively, mimicking gene expression in acute phase reactions, such as those in response to bacterial infection or body injury (*20*). We found that this gene activation was inhibited specifically by 2-ME, but once the gene had been activated, 2-ME no longer inhibited its transcription, suggesting that oxidation of the sulfhydryl group of a fat body protein is a prerequisite for *Sarcophaga* lectin gene activation (*20*).

In this study, we further investigated *Sarcophaga* lectin gene activation in this system. AdoHcy is known to be a potent inhibitor of transmethylation (*35*). We induced AdoHcy synthesis in fat bodies by culturing them in medium containing Ado and HcyTL (*33, 34*). Under these conditions, *Sarcophaga* lectin gene expression, but not HSP70 gene expression, was inhibited selectively and the cellular transmethylation reaction was inhibited markedly. Thus, transmethylation reaction seems to be also a prerequisite for the activation of *Sarcophaga* defense protein genes.

We found that the transmethylation reaction of certain fat body proteins was also inhibited significantly when fat bodies were cultured in the presence of 2-ME. Therefore, inhibition of *Sarcophaga* lectin gene expression by 2-ME is a complex reaction and may not be simply due to prevention of the oxidation of sulfhydryl group of a fat body protein. As 2-ME is known to be methylated by the transmethylation reaction and detoxicated in the cell (*30-32*), the fat bodies may become deficient in the methyl donor (*S*-adenosyl-L-methionine) when they are cultured in the presence of 2-ME, due to preferential use of the donor to detoxicate 2-ME. Consequently, the transmethylation reaction of a certain fat body protein essential for defense protein gene activation is blocked, resulting in suppression of their activation. This transmethylation reaction may be needed only for activation of defense protein genes.

It has been suggested that *Rel* family transcription factors participate in insect defense protein gene activation (*38*). The NF-κB-binding motif in the 5′-upstream region of the *Sarcophaga* lectin gene was found to be essential for accurate transcription initiation of this gene *in vitro* and we assume that the 59-kDa protein that binds specifically to this sequence participates in this reaction (*19*). This protein is likely to be a *Rel* family transcription factor, such as NF-κB. Law *et al.* showed that 5′-methylthioadenosine, an inhibitor of several *S*-adenosyl-methionine dependent methylation reaction, inhibited the lipopolysaccharide induced NF-κB activation in mouse 70Z/3 pre-B lymphocytes (*39*).

The cellular concentration of GSH is known to be crucial for NF-κB activation (*22*), and we have demonstrated that the GSH level of the fat body decreases significantly when *Sarcophaga* larval body wall is injured and when fat bodies are cultured *in vitro* in BIS (*21*). However, nothing is known of the mechanism that regulates the cellular GSH level in the fat body. We assume that methylation of a certain fat body protein is needed for the induction of GSH metabolism in the fat body that we reported previously (*21*), and this methylation is preferentially inhibited by 2-ME. This transmethylation reaction may be triggered by integumental injury and/or bacterial infection of *Sarcophaga* larvae. The same transmethylation reaction appeared to be activated when a fat body was simply cultured in BIS.

REFERENCES

1 Boman, H G and Hultmark, D. (1987) Cell-free immunity in insects. *Annu. Rev. Microbiol.* **41**, 103-126
2. Steiner, H., Hultmark, D , Engstrom, A., Bennich, H , and Boman, H.G. (1981) Sequence and specificity of two antibacterial proteins involved in insect immunity *Nature* **292**, 246-248
3. Hultmark, D , Engstrom, A., Andersson, K , Steiner, H., Bennich, H., and Boman, H.G. (1983) Insect immunity. Attacins, a family of antibacterial proteins from *Hyalophora cecropia* *EMBO J* **2**, 571-576
4. Okada, M. and Natori, S. (1983) Purification and characterization of an antibacterial protein from hemolymph of *Sarcophaga peregrina* (flesh-fly) larvae *Biochem. J.* **211**, 727-734
5. Ando, K , Okada, M., and Natori, S (1987) Purification of Sarcotoxin II, antibacterial proteins of *Sarcophaga peregrina* (flesh-fly) larvae. *Biochemistry* **26**, 226-230
6 Matsuyama, K. and Natori, S. (1988) Purification of three

antibacterial proteins from the culture medium of NIH-Sape-4, an embryonic cell line of *Sarcophaga peregrina*. *J. Biol. Chem.* **263**, 17112-17116
7. Dimarcq, J.L., Keppi, E., Dunbar, B., Lambert, J., Reichhart, J.M., Hoffmann, D., Rankine, S.M., Fothergill, J.E., and Hoffmann, J.A. (1988) Insect immunity. Purification and characterization of a family of novel inducible antibacterial proteins from immunized larvae of the dipteran *Phormia terranovae* and complete amino-acid sequence of the predominant member, diptericin A. *Eur. J. Biochem.* **171**, 17-22
8. Lambert, J., Keppi, E., Dimarcq, J.L., Wicker, C., Reichhart, J.M., Dunbar, B., Lepage, P., van Dorsselaer, A., Hoffmann, J., Fothergill, J., and Hoffmann, D. (1989) Insect immunity: Isolation from immune blood of the dipteran *Phormia terranovae* of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. *Proc. Natl. Acad. Sci. USA* **86**, 262-266
9. Komano, H., Mizuno, D., and Natori, S. (1980) Purification of lectin induced in the hemolymph of *Sarcophaga peregrina* larvae on injury. *J. Biol. Chem.* **255**, 2919-2924
10. Jomori, T. and Natori, S. (1991) Molecular cloning of cDNA for lipopolysaccharide-binding protein from the hemolymph of the American cockroach, *Periplaneta americana*. *J. Biol. Chem.* **266**, 13318-13323
11. Takahashi, H., Komano, H., and Natori, S. (1986) Expression of the lectin gene in *Sarcophaga peregrina* during normal development and under conditions where the defense mechanism is activated. *J. Insect Physiol.* **32**, 771-779
12. Matsumoto, N., Okada, M., Takahashi, H., Qu, X.-M., Nakajima, Y., Nakanishi, Y., Komano, H., and Natori, S. (1986) Molecular cloning of a cDNA and assignment of the C-terminal of Sarcotoxin IA, a potent antibacterial protein of *Sarcophaga peregrina*. *Biochem. J.* **239**, 717-722
13. Matsuyama, K. and Natori, S. (1988) Molecular cloning of cDNA for Sapecin and unique expression of the Sapecin gene during the development of *Sarcophaga peregrina*. *J. Biol. Chem.* **263**, 17117-17121
14. Sun, S.-C., Lindstrom, I., Lee, J.-Y., and Faye, I. (1991) Structure and expression of the attacin genes in *Hyalophora cecropia*. *Eur. J. Biochem.* **196**, 247-254
15. Engstrom, Y., Kadalayil, L., Sun, S.-C., Samakovlis, C., Hultmark, D., and Faye, I. (1993) κB-like motifs regulate the induction of immune genes in *Drosophila*. *J. Mol. Biol.* **232**, 327-333
16. Baeuerle, P.A. (1991) The inducible transcription activator NF-κB: Regulation by distinct protein subunits. *Biochim. Biophys. Acta* **1072**, 63-80
17. Schmitz, M.L., Henkel, T., and Baeuerle, P.A. (1991) Proteins controlling the nuclear uptake of NF-κB, Rel and dorsal. *Trends Cell Biol.* **1**, 130-137
18. Sun, S.-C. and Faye, I. (1992) Affinity purification and characterization of CIF, an insect immunoresponsive factor with NF-κB-like properties. *Comp. Biochem. Physiol.* **103B**, 225-233
19. Kobayashi, A., Matsui, M., Kubo, T., and Natori, S. (1993) Purification and characterization of a 59-kilodalton protein that specifically binds to NF-κB binding motifs of the defense protein genes of *Sarcophaga peregrina* (the flesh fly). *Mol. Cell. Biol.* **13**, 4049-4056
20. Sugiyama, H. and Natori, S. (1991) A novel mechanism of *Sarcophaga* lectin gene expression. Possible involvement of oxidation of the sulfhydryl group of a fat-body protein. *Eur. J. Biochem.* **200**, 495-500
21. Sugiyama, H. and Natori, S. (1994) Induction of glutathione metabolism in the fat body of *Sarcophaga peregrina* by integumental injury. *J. Biochem.* **116**, 1171-1175
22. Staal, F.J.T., Roederer, M., Herzenberg, L.A., and Herzenberg, L.A. (1990) Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **87**, 9943-9947
23. Thomas, P.S. (1980) Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77**, 5201-5205
24. Takahashi, H., Komano, H., Kawaguchi, N., Kitamura, N., Nakanishi, S., and Natori, S. (1985) Cloning and sequencing of cDNA of *Sarcophaga peregrina* humoral lectin induced on injury of the body wall. *J. Biol. Chem.* **260**, 12228-12233
25. Ando, K. and Natori, S. (1988) Molecular cloning, sequencing, and characterization of cDNA for Sarcotoxin IIA, an inducible antibacterial protein of *Sarcophaga peregrina* (flesh-fly). *Biochemistry* **27**, 1715-1721
26. Matsumoto, N., Sekimizu, K., Soma, G., Ohmura, Y., Andoh, T., Nakanishi, Y., Obinata, M., and Natori, S. (1985) Structural analysis of a developmentally regulated 25-kDa protein gene of *Sarcophaga peregrina*. *J. Biochem.* **97**, 1501-1508
27. Ingolia, T.D., Craig, E.A., and McCarthy, B.J. (1980) Sequence of three copies of the gene for the major *Drosophila* heat shock induced protein and their flanking regions. *Cell* **21**, 669-679
28. Gordon, R.K., Miura, G.A., Alonso, T., and Chiang, P.K. (1987) *S*-Adenosylmethionine and its sulfur metabolites. *Methods Enzymol.* **143**, 191-195
29. Stock, J.B., Clarke, S., and Koshland, D.E., Jr. (1984) The protein carboxylmethyltransferase involved in *Escherichia coli* and *Salmonella typhimurium* chemotaxis. *Methods Enzymol.* **106**, 310-321
30. Bremer, J. and Greenberg, D.M. (1961) Enzymic methylation of foreign sulfhydryl compounds. *Biochim. Biophys. Acta* **46**, 217-224
31. Weisiger, R.A. and Jakoby, W.B. (1979) Thiol *S*-methyltransferase from rat liver. *Arch. Biochem. Biophys.* **196**, 631-637
32. Weisiger, R.A. and Jakoby, W.B. (1981) Thiol *S*-methyltransferase. *Methods Enzymol.* **77**, 257-262
33. de la Haba, G. and Cantoni, G.L. (1959) The enzymatic synthesis of *S*-adenosyl-L-homocysteine from adenosine and homocysteine. *J. Biol. Chem.* **234**, 603-608
34. Schatz, R.A., Wilens, T.E., and Sellinger, O.Z. (1981) Decreased transmethylation of biogenic amines after *in vivo* elevation of brain *S*-adenosyl-L-homocysteine. *J. Neurochem.* **36**, 1739-1748
35. Hurwitz, J., Gold, M., and Anders, M. (1964) The enzymatic methylation of ribonucleic acid and deoxyribonucleic acid. *J. Biol. Chem.* **239**, 3474-3482
36. Barten, D.M. and O'Dea, R.F. (1990) The function of protein carboxylmethyltransferase in eucaryotic cells. *Life Sci.* **47**, 181-194
37. Bremer, J. and Greenberg, D.M. (1961) Methyl transferring enzyme system of microsomes in the biosynthesis of lecithin (phosphatidylcholine). *Biochim. Biophys. Acta* **46**, 205-216
38. Ip, Y.T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., Crespo, S.G., Tatei, K., and Levine, M. (1993) *Dif* a *dorsal*-related gene that mediates an immune response in *Drosophila*. *Cell* **75**, 753-763
39. Law, R.E., Stimmel, J.B., Damore, M.A., Carter, C., Clarke, S., and Wall, R. (1992) Lipopolysaccharide-induced NF-κB activation in mouse 70Z/3 pre-B lymphocytes is inhibited by mevinolin and 5′-methylthioadenosine: Roles of protein isoprenylation and carboxyl methylation reactions. *Mol. Cell. Biol.* **12**, 103-111