Heat stress induced enhancement of heat shock protein gene activity in the honey bee (*Apis mellifera*)

D. W. Severson[a], E. H. Erickson Jr[b], J. L. Williamson[a] and J. M. Aiken[a]

[a]*Department of Veterinary Science, University of Wisconsin, Madison (Wisconsin 53706, USA), and* [b]*USDA-ARS, Carl T. Hayden Bee Research Center, 2000 East Allen Road, Tucson (Arizona 85719, USA)*
Received 22 September 1989; accepted 12 January 1990

Summary. We employed in vitro translation of mRNA and product separation using SDS-PAGE to examine the heat-shock response of the worker honey bee. Increases in the levels of 6 translatable RNA populations were observed following heat stress. The greatest response was observed among bees aged 9 days. Slight levels of induction of 70 and 82 kDa heat shock proteins were evident among bees taken directly from the colony.
Key words. Heat shock protein; stress; in vitro translation; gene regulation; *Apis mellifera*.

Exposure to elevated temperatures and a wide variety of environmental stresses induces the production of a small number of highly conserved proteins in cultured cells and whole organisms: these proteins have been designated as the heat-shock proteins or hsps. The response appears to be universal among all organisms examined including such diverse organisms as *Escherichia coli*, *Drosophila melanogaster*, *Zea mays*, and man[1–3]. Some of the heat shock genes have been highly conserved during evolution: the hsp70 protein from man, for example, is 73% identical to that of *Drosophila*[4]. Induction of hsp synthesis occurs at different temperatures in different organisms: induction temperatures typically correspond to the upper portion of the natural growth range of each organism[2].

The highly social structure of the honey bee (*Apis mellifera*) colony includes a unique adaptation among poikilotherms: although individual bees exhibit poor thermoregulatory capabilities, intact clusters of several thousand individuals exhibit excellent thermoregulation over a broad continuum of temperature extremes[5,6]. The cluster center can be maintained at ca 33–34 °C, regardless of the ambient temperature. The honey bee cluster has, therefore, been viewed as a superorganism and a functional homeotherm[7].

Since in other arthropods, such as *Drosophila*, normal cluster temperatures would be sufficient to induce hsp production[8], we questioned whether honey bees have evolved a higher threshold for hsp induction or whether some level of natural induction would be evident within the normal confines of the active cluster. Here we describe the heat-shock response of the worker honey bee. We employed in vitro translation of mRNA and product separation using SDS-PAGE to examine transcriptional activity in bees: 1) taken directly from the colony; 2) removed from the colony and acclimated to the ambient temperature; and 3) removed from the colony and exposed to a heat stress.

Materials and methods

Newly emerged workers within a colony were marked (with a distinct paint dot on their thorax) at intervals which provided defined-age cohorts of 0, 9, and 27 days. Two colonies were examined in this manner. Also examined were broadly defined age-groups of workers from several additional colonies: 1) newly emerged; 2) young bees removed from the brood nest area; and 3) older bees obtained at the hive entrance. Test bees were allowed free flight and normal participation in colony activities until just prior to a given experiment.

In each experiment, representative age cohorts of bees were collected from a colony and divided into 3 treatments (n = 10 bees per treatment). Bees representing the first treatment (A) were immediately frozen in liquid N_2 and stored at − 80 °C. This group therefore reflects normal within-colony levels of transcriptional activity. Bees representing the second (B) and third (C) treatments were placed in screen cages and transported directly to the laboratory. Bees in treatment B were placed on a laboratory bench (T = 26 °C) for about 4 h, frozen in liquid N_2 and stored at − 80 °C. This group therefore reflects levels of transcriptional activity in isolated bees at ambient temperatures. Bees in treatment C were placed in an incubator at 42 °C for about 4 h, frozen in liquid N_2 and stored at − 80 °C. A 4-h treatment period was selected based upon kinetics of the hsp response in *Drosophila*: it provided a high probability for a maximum hsp response[8] (treatment C) and conversely, it provided an adequate period for inactivation of hsp mRNAs produced at the normal cluster temperature of 33–34 °C[9] (treatment B).

RNA was isolated from pooled samples of 3 bees per treatment as previously described[10]. Briefly, samples were homogenized in phenol and a lysis solution containing 0.5% SDS, followed by standard phenol/chloroform extraction. Nucleic acids were initially precipitated in ethanol and RNA subsequently selectively precipitated in 3 M ammonium acetate. 5 µg of RNA from each sample were translated in vitro in the presence of ^{35}S-methionine (Amersham) using a rabbit reticulocyte system (Promega). Constant volumes of the translation mixture were compared using one- and two-dimensional SDS-polyacrylamide gel electrophoresis as previously described[10]. Autoradiography was performed at − 80 °C with an intensifying screen.

Results and discussion

Exposure of 0-, 9-, and 27-day-old worker bees to 42 °C for 4 h resulted in an increase in levels of at least 6 translatable RNA populations (fig. 1, compare heat-stressed bees, lane C, to bees held at ambient temperature, lane B, in each age group). The molecular weights of these translation products are similar to hsps observed in heat-shocked *Drosophila*[8]. Similar heat-shock-specific increases in higher molecular weight (70 and 82 kDa) translation products were observed across all age groups. Heat-shock-specific increases in lower molecular weight translation products (23, 27, 33 and 36 kDa) were always most evident in 9-day-old bees. This age-specific variability in hsp induction is not inconsistent with well-known aspects of worker bee nutrition. That is, workers do not begin consuming pollen (the sole protein source of the honey bee) until several hours after adult eclosion and subsequently shift by 8–10 days post-eclosion to a predominantly carbohydrate diet[11]. Newly emerged and older workers therefore exhibit lower nitrogen contents, suggesting the potential for decreased transcriptional and translational activity. Investigations with *Drosophila* have demonstrated that transcriptional activity is very similar in very young and older individuals and that a more complex pattern of transcriptional activity is observed in middle-aged individuals[12].

Our results further suggest that slight levels of hsp induction of some hsps occur within the normal colony environment. The higher molecular weight (70 and 82 kDa) translation products associated with heat-stress (fig. 1, lane C) are evident, albeit at lower levels, among bees taken directly from the colony (fig. 1, lane A), but are clearly reduced among bees held at ambient temperature (fig. 1, lane B). Similar increases in the lower molecular weight translation products are not evident. This phenomenon is not surprising as the role of low molecular weight hsps in the acquisition of thermotolerance is uncertain[3]. Within-colony hsp induction was clearly evident following two-dimensional gel electrophoresis of translation products (fig. 2). Also, as in *Drosophila*[8], the honey bee hsp70 gene family is multigenic: we observed an increase in relative intensity of at least 4 spots in the 68–70 kDa category following heat stress. Physiological

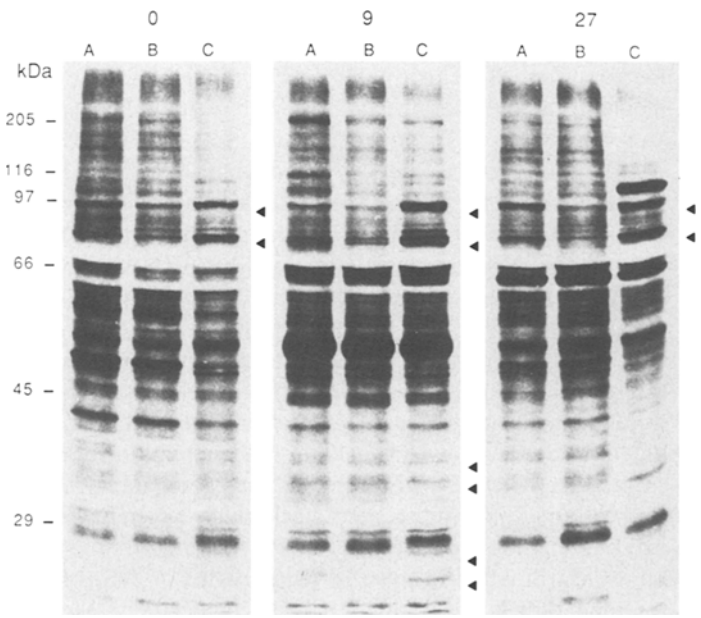

Figure 1. Typical autoradiograms of SDS-12% polyacrylamide gels showing in vitro translation products observed among adult worker bees aged 0, 9, and 27 days. *A* bees taken directly from the colony; *B* bees acclimated to ambient temperature for 4 h; *C* bees exposed to 42 °C for 4 h. Arrowheads delineate heat stress inducible translation products. Molecular weight markers (kDa) are indicated.

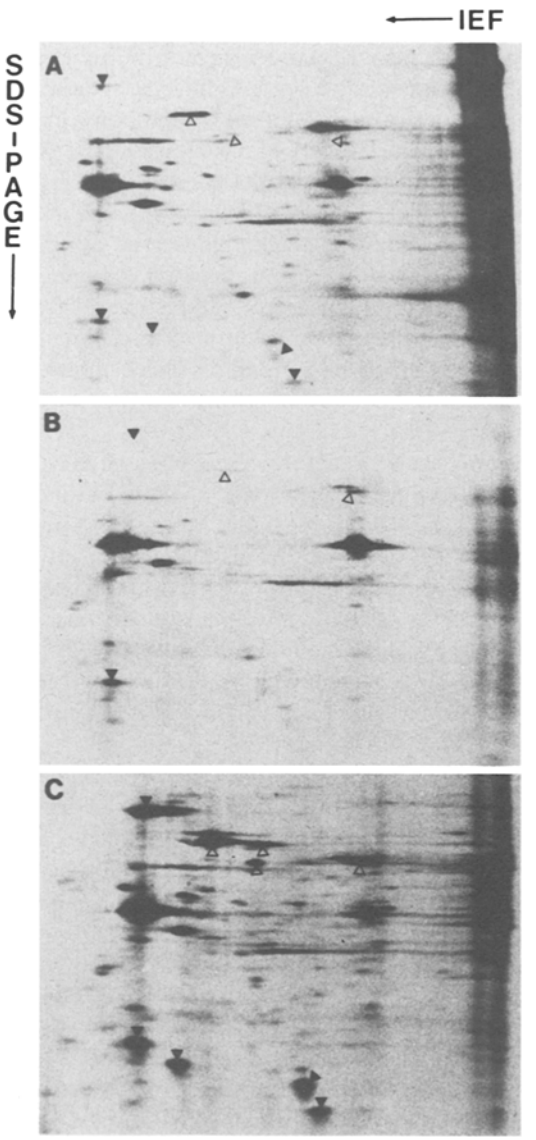

Figure 2. Typical autoradiograms of two-dimensional separation of in vitro translation products from worker bees aged 9 days: *A* taken directly from the colony; *B* acclimated to ambient temperature for 4 h; and *C* exposed to 42 °C for 4 h. Isoelectric focusing was conducted on a pH 5–7 gradient. Second-dimension electrophoresis was in SDS-12% acrylamide slab gels. Arrowheads delineate heat stress inducible translation products. Open arrowheads indicate members of the hsp70 gene family.

studies by Free and Spencer-Booth [13] support our observations of within-colony hsp induction: survivorship of bees exposed to 45–47 °C is higher among bees previously acclimated to normal colony temperatures (35 °C) than bees initially held at ambient temperature (20 °C).

We hypothesize that slight hsp induction within the normal colony may provide an adaptive mechanism for resisting the potential adverse effects of colony overheating. Although the functional significance of the heat-shock response is presently uncertain, the response may be a ubiquitous mechanism for maintaining cellular homeostasis. There is clear evidence at both the cellular and organismal levels that induction of hsp synthesis is frequently correlated with the acquisition of thermotolerance. That is, an initial exposure to mild heat-shock conditions confers increased survivorship to previously lethal temperatures [2, 3, 14, 15]. Within-colony hsp induction (and subsequent acquisition of thermotolerance) may be important to honey bees not only during warm weather, but also in cold climates as colony survival is dependent upon heat production by individual bees (primarily by microvibration of flight muscles) within the cluster [16]. Thoracic temperatures of these bees at times undoubtedly exceed 40 °C in order to maintain cluster temperatures of 33–34 °C. Core temperatures of 40.5 °C have been reported under cold weather conditions [5].

There is also a growing body of evidence that hsps can be induced by a variety of other stress treatments [15]. Agents that do induce hsp synthesis also frequently convey thermotolerance to the organism [1–3]. Most importantly, cross-resistance among various inducing agents is often observed: heat-shock, for example, induces tolerance to ethanol, anoxia and several other forms of stress [2]. If hsp induction in the honey bee proves to be associated with various environmental stimuli, observations of levels of induction may provide a vehicle for monitoring the effects of various stress-inducing agents on colony dynamics.

1 Schlesinger, M. J., Ashburner, M., and Tissieres, A., (eds), Heat Shock from Bacteria to Man. Cold Spring Harbor, New York 1982.
2 Lindquist, S., A. Rev. Biochem. *55* (1986) 1151.
3 Lindquist, S., and Craig, E. A., A. Rev. Genet. *22* (1988) 631.
4 Hunt, C., and Morimoto, R. I., Proc. natl Acad. Sci. USA *82* (1985) 6455.
5 Owens, C. D., USDA-ARS Tech. Bull. 1429 (1971) 1.
6 Southwick, E. E., J. comp. Physiol. *156B* (1985) 143.
7 Southwick, E. E., Comp. Biochem. Physiol. *75A* (1983) 641.
8 Ashburner, M., and Bonner, J. J., Cell *17* (1979) 241.
9 DiDomenico, B. J., Bugaisky, G. E., and Lindquist, S., Proc. natl Acad. Sci. USA *79* (1982) 6181.
10 Severson, D. W., Williamson, J. L., and Aiken, J. M., Insect Biochem. *19* (1989) 215.
11 Haydak, M. H., A. Rev. Ent. *15* (1970) 143.
12 Fleming, J. E., Quattrocki, E., Latter, G., Miquel, J., Marcuson, R., Zuckerkandl, E., and Bensch, K. G., Science *231* (1986) 1157.
13 Free, J. B., and Spencer-Booth, Y., Ent. exp. appl. *5* (1962) 249.
14 Neidhardt, F. C., VanBogelen, R. A., and Vaughn, V., A. Rev. Genet. *18* (1984) 295.
15 Nover, L., (ed.), Heat Shock Response of Eukaryotic Cells. Springer-Verlag, New York 1984.
16 Esch, H., Z. vergl. Physiol. *43* (1960) 305.

0014-4754/90/070737-03$1.50 + 0.20/0

Production of active and passive anaphylactic shock in the WBB6F$_1$ mouse, a mast cell-deficient strain

A. Arimura, M. Nagata, A. Watanabe, K. Nakamura, M. Takeuchi and M. Harada*

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 (Japan)
Received 20 November 1989; accepted 9 January 1990

Summary. The role of mast cells in active and passive anaphylactic shock was examined using the WBB6F$_1$ mouse, a genetically mast cell-deficient strain. Lethal anaphylactic shock occurred at high incidence rates in mice actively sensitized to bovine serum albumin (BSA). The reaction was specific to BSA since the shock could not be elicited by human or guinea pig serum albumin in these animals. Lethal shock could be prevented by CV-3988 but not by cyproheptadine, which suggests that the shock is mediated by PAF but not by histamine and serotonin. Similarly, lethal shock was provoked by homologous antigens in mice which had been passively sensitized with allogeneic anti-benzylpenicilloyl (BPO) IgG$_1$ monoclonal antibody or with allogeneic or xenogeneic anti-BSA antiserum, but not in those sensitized with allogeneic anti-BPO IgE monoclonal antibody. These findings suggest that mast cells are not necessarily required for anaphylactic shock in the mouse.
Key words. WBB6F$_1$ mouse; anaphylactic shock; IgE antibody; IgG$_1$ antibody; xenogeneic antibody; mast cell-deficiency.

Type I hypersensitivity reactions, such as anaphylactic shock and passive cutaneous anaphylaxis (PCA), is caused by histamine, serotonin or other chemical mediators released by antigen-antibody interaction from mast cells and basophils [1]. The role of mast cells would be particularly important in the mouse since, in general, few basophils are present in mouse blood [2]. However, it has recently been found that anaphylactic shock in mice is