

Heat Sensitivity, Thermotolerance, and Profile of Heat Shock Protein Synthesis of Human Myelogenous Leukemias¹

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ABSTRACT

In anticipation of using single or fractionated hyperthermia treatment in *ex vivo* purging of leukemic bone marrow in the clinic, we have compared the hyperthermic sensitivity, kinetics of thermotolerance, and heat-shock protein synthesis in three human myelogenous leukemic cell lines. In terms of heat sensitivity, the chronic myelogenous leukemic cell line K562 was found to be the most resistant. The D_{50} s of the 43, 44, and 45°C heat survival curves were 22, 13, and 6 min, respectively. HL-60 and KG-1, both acute myelogenous leukemic lines, however, were found to be several fold more sensitive to the cytotoxic effects of heat. The D_{50} s of the 43, 44, and 45°C heat survival curves for HL-60 were 7.6, 5.6, and 2 min and for KG-1 were 5.7, 4.5, and 1.7 min, respectively.

All cell lines developed thermotolerance. However, K562 developed more tolerance which lasted for longer times. For K562 cells at priming heat doses of 45°C/10 min, 42°C/2 h, or 41°C/2 h thermotolerance was maximum at 4 to 6 h and began to decay at 24 h. HL-60 and KG-1 cells showed some thermotolerance at the priming doses of 45°C/5 min or 42°C/30 min and had fully decayed by 24 h. K562 cells synthesized M_r 70,000 heat shock protein for over 24 h following the 45°C/10 min heat shock, while HL-60 and KG-1 synthesized M_r 70,000 heat shock protein for 2-4 h for the same amount of cell kill. These studies suggest that most human leukemias may be extremely sensitive to the cytotoxic effects of heat, and *in vitro* purging of leukemias from bone marrow specimens by heat needs to be further studied both by *in vitro* and *in vivo* model systems.

INTRODUCTION

WBH³ is currently being used in the treatment of refractory leukemias and lymphomas (1, 2). Recently we and others have performed studies related to the hyperthermic sensitivity and kinetics of thermotolerance induction and decay of normal murine and human granulocyte-macrophage progenitors (3-7). WBH or *in vitro* purging of leukemic bone marrow by heat prior to autologous bone marrow transplantation in the treatment of leukemias and lymphomas would be most beneficial if the majority of human leukemias would be more sensitive to hyperthermia than normal bone marrow progenitors. This increased hyperthermic sensitivity of leukemias would ensure total eradication of residual leukemias.

Previous studies have indicated that some leukemic cells are more sensitive to heat than normal bone marrow stem cells (8-11). However, systematic studies related to hyperthermic response of human myeloid leukemias are lacking. Furthermore, no studies have been performed examining the thermotolerance

of human myeloid leukemias. Studies related to the kinetics of thermotolerance induction and decay could lead to the use of *in vitro* purging of leukemias by fractionated heat treatments, or one can use fractionated WBH and take advantage of systemic tolerance (12, 13) and tolerance in other normal tissues. In the studies presented here, hyperthermic response and kinetics of thermotolerance induction and decay of 3 human myeloid leukemias have been compared. The kinetics of thermotolerance has also been correlated with the synthesis of heat shock proteins.

MATERIALS AND METHODS

Cell Lines. All cell lines were obtained from the American Type Culture Collection. The K562 cell line is a chronic myelogenous leukemia in terminal blast crisis and is highly undifferentiated (14). The HL-60 line is an acute promyelocytic leukemia and can differentiate to granulocyte-monocyte series (15). The KG-1 cell line is also an acute myelogenous leukemia, and it is highly undifferentiated (16). K562 and HL-60 cells can form colonies on semisolid medium and do not need any colony-stimulating factor. However, the KG-1 line forms colonies on semisolid medium only in the presence of granulocyte-macrophage colony-stimulating factor (16). All cells were maintained in IMDM supplemented with 20% FCS. The doubling times for K562, HL-60, and KG-1 cells were 14, 48, and 72 h, respectively.

***In Vitro* Heating and Incubation.** Cells were grown in IMDM supplemented with 20% FCS. For heating, 2 ml of 1 to 2×10^6 cells per ml were suspended in the above medium in 15-ml centrifuge tubes and heated as previously described (5). The immersion depth was approximately 4 cm above where the cell suspension ended, and the t_{90} of heat treatment was 2 min.

Cell Survival Assays. After appropriate treatment, cells were centrifuged and plated in 35-mm Petri dishes. The growth medium contained α -MEM, 10% FCS, and 0.3% Noble agar (17). KG-1 cells were supplemented with 150 units of rGM-CSF (18), generous gift of the Genetics Institute of Boston. Plating efficiencies for K562, HL-60, and KG-1 were 20 to 30%, 10 to 20%, and 5 to 15%, respectively. All lines were incubated at 37°C, 95% humidity, 5% CO₂ for 10 to 14 days. Only colonies of 50 or more cells were counted. All experiments were repeated at least twice. The error bars represent the mean \pm SD of at least 3 experiments. In all thermotolerant experiments, the survival values have been adjusted for initial cell killing of the priming heat doses.

Labeling and Electrophoresis. Approximately 2×10^6 cells were suspended in methionine-free MEM and labeled for 2 h at 37°C with [³⁵S]methionine (40 μ Ci/ml; specific activity, >600 Ci/mmol; Amersham/Searle Corp.). Cells were then washed with cold phosphate-buffered saline and lysed in sodium dodecyl sulfate sample buffer for one-dimensional gel electrophoresis as previously described (19).

RESULTS

Heat Sensitivity of Human Myeloid Leukemias. Recently we examined the heat response of normal human granulocyte-macrophage progenitors. In the studies presented here, 3 different myeloid leukemias were tested for their heat response to compare their heat sensitivity with that of normal human CFU-GM. All lines were heated at 41°C-45°C for increasing lengths

Received 8/1/88; revised 11/15/88; accepted 1/17/89.

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¹ This work was supported by NIH Grant CA 33572.

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³ The abbreviations used are: WBH, whole-body hyperthermia; HSP, heat shock protein; CFU-GM, colony-forming unit-granulocyte and macrophages; α -MEM, enriched minimal essential medium; FCS, fetal calf serum; IMDM, Iscove's modified Dulbecco's medium; rGM-CSF, recombinant granulocyte-macrophage colony-stimulating factor.

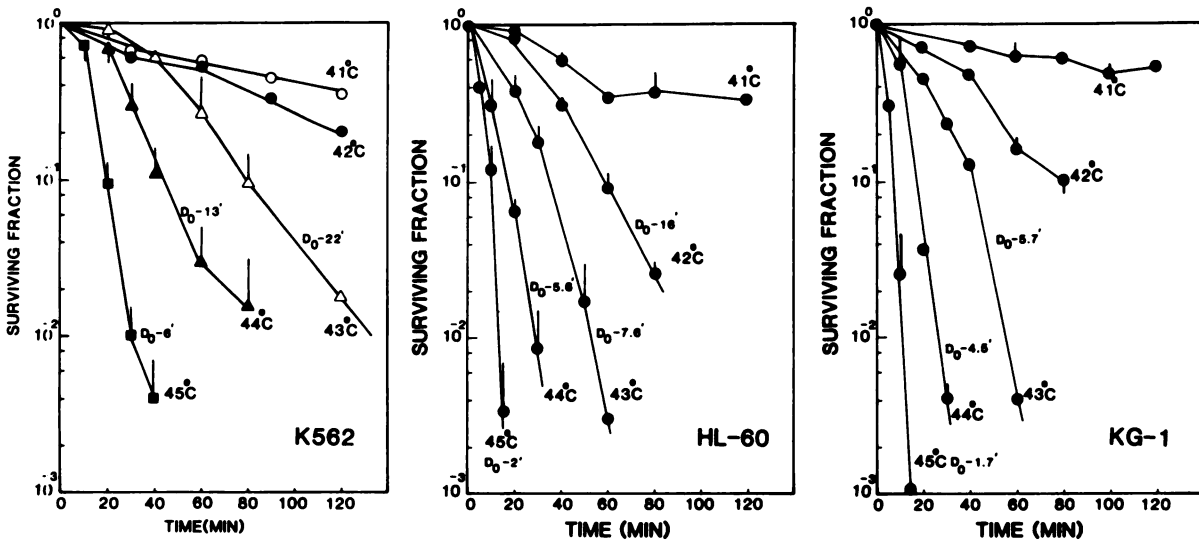


Fig. 1. Heat response of human myeloid leukemia. Cells were heated at 41–45°C for an increasing length of time and were then plated for colony formation on soft agar.

of time. K562 was the most resistant line (Fig. 1). As the results in Table 1 indicate, in terms of D_{0s} , the K562 cells' response to heat was virtually the same as that of normal human CFU-GM reported previously. HL-60 and KG-1 cells, however, were much more sensitive to heat (Fig. 1; Table 1). Both cell lines showed dramatic reduction in the D_0 at 43, 44, and 45°C.

Thermotolerance of Human Myeloid Leukemias. The following experiments were performed to investigate the kinetics of thermotolerance induction and decay of the leukemic cell lines. K562 cells were given a priming heat dose of 45°C/10 min, 41°C/2 h, or 42°C/2 h (Figs. 2 and 3), returned back to 37°C for up to 24 h, and then challenged with 45°C heat shock. K562 cells were tolerant in all cases 2 h following the priming heat doses or immediately following the priming heat dose of 41°C or 42°C. Thermotolerance was maximum at 4 to 6 h and began to decay at 24 h. At 41°C/2 h priming heat shock, however, thermotolerance was already maximum and decayed almost entirely at 24 h. HL-60 and KG-1 cells also developed thermotolerance, but they developed less tolerance and showed a faster decay rate (Figs. 4 and 5). At priming doses of 45°C/5 min or 42°C/30 min, both lines showed some tolerance at 4 to 6 h with tolerance decaying completely by 24 h. A comparison of the rate of thermotolerance induction and decay of normal human CFU-GM and the three leukemias is shown in Fig. 6. In order to make a comparison between the amount of thermotolerance induction, the time tolerance reaches its maximum, and the time thermotolerance begins to decay, cells were heated so that there was approximately a 50% reduction in cell survival after the priming heat doses. As the results in Fig. 6 indicate, HL-60 and KG-1 cells develop less tolerance and at the slower rate. Furthermore, thermotolerance decays at a faster rate in HL-60 and KG-1 cells. CFU-GM and K562 cells show a similar kinetics of thermotolerance induction and decay.

Heat shock protein synthesis in K562, HL-60, and KG-1 cells following the priming doses of 45°C/10 min or 42°C/30 min is shown in Fig. 7. M_r 87,000 HSP synthesis was evident in all control and heat-shocked cells. K562 cells synthesized M_r 70,000 HSP for over 24 h, while HL-60 and KG-1 cells synthesized M_r 70,000 HSP for only 2 to 4 h. This differential rate of

Table 1 Thirty-seven % dose slope (D_0) of normal human CFU-GM and leukemic cells K562, KG-1, and HL-60

The units of D_0 values reported are in minutes.

| Temperature | CFU-GM | K562 | KG-1 | HL-60 |
|-------------|--------|------|------|-------|
| 43°C | 23 | 22 | 5.7 | 7.6 |
| 44°C | 12 | 13 | 4.5 | 5.6 |
| 45°C | 5 | 6 | 1.7 | 2 |

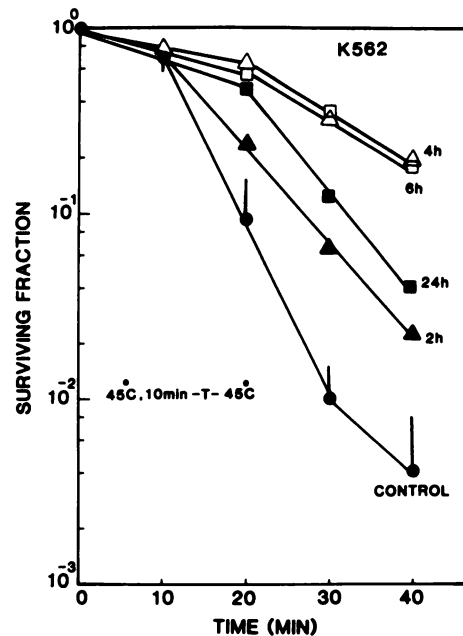
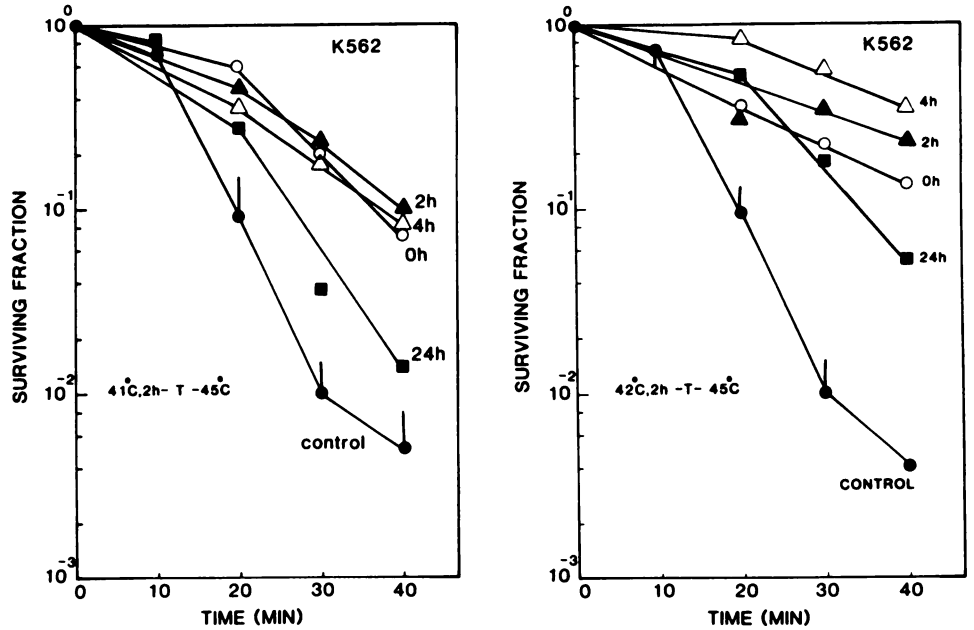


Fig. 2. Thermotolerance in K562 cells at a priming heat dose of 45°C/10 min. K562 cells were given a priming heat dose of 45°C/10 min and after 2, 4, 6, and 24 h of incubation at 37°C, cells were challenged with 45°C heat shock and plated for colony formation in soft agar.

M_r 70,000 HSP synthesis in heat-resistant and -sensitive cell lines could result in the differential accumulation of this protein in cells and subsequently result in the different pattern of tolerance seen in these cells.

Fig. 3. Thermotolerance in K562 cells at a priming heat dose of 41°C/2 h and 42°C/2 h. K562 cells were given a priming heat dose of 41°C/2 h or 42°C/2 h. After 2, 4, and 24 h of incubation at 37°C, cells were challenged with 45°C heat shock and plated for colony formation in soft agar.



DISCUSSION

There is some evidence that leukemic cells may be more sensitive to cytotoxic effects of heat than their normal bone marrow progenitors (8-11). However, such information is limited in relation to variety of human leukemias and normal bone marrow progenitors.

Recently, we studied the heat response of normal human CFU-GM and their ability to develop thermotolerance (5). As the results in Table 1 indicate, the human CFU-GM showed a D_0 value of 23, 12, and 5 min for 43, 44, and 45°C, respectively. In this study K562, a human chronic myelogenous leukemic cell line, showed similar heat response as that of normal CFU-GM. The two acute myelogenous leukemic cells, HL-60 and KG-1, however, were 3 times more sensitive (in terms of the D_0) to hyperthermia. The reason for the differences in the

thermal response of different leukemic cells is not known. The differentiation state, chromosomal abnormalities, presence of a variety of oncogenes, and finally the type of leukemia may contribute to this differential heat response in leukemic cells (16, 20-22). More studies need to be done to confirm such possibilities. What is clear from these studies, however, is that this increased heat sensitivity of some leukemic cells as compared to normal bone marrow progenitors may be used to purge residual leukemic cells from bone marrow specimens.

Leukemic cells and normal CFU-GM also differ in terms of kinetics of thermotolerance decay. K562 cells show a similar rate of thermotolerance induction and decay as that of normal CFU-GM, while HL-60 and KG-1 cells show a slower rate of tolerance induction and a faster decay rate. HL-60 and KG-1 cells also develop a lesser amount of thermotolerance. This differential rate of thermotolerance induction and decay be-

Fig. 4. Thermotolerance in HL-60 cells following a priming heat dose of 45°C/5 min or 42°C/30 min. Cells were given a priming heat dose of 45°C/5 min or 42°C/30 min. After 4, 6, and 24 h of incubation at 37°C, cells were challenged at 45°C or 43°C and were plated for colony formation in soft agar.

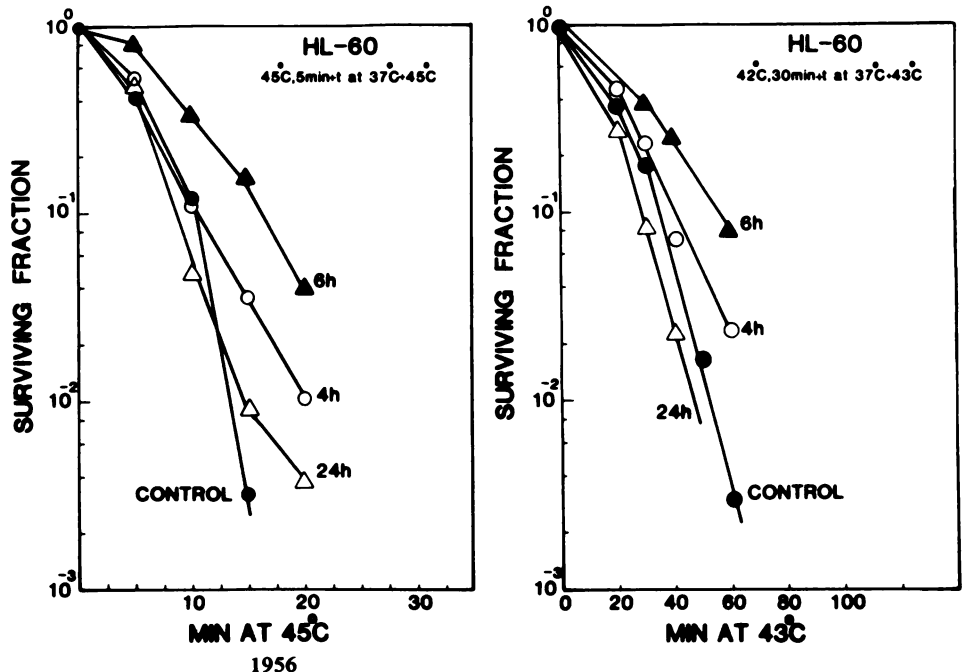


Fig. 5. Thermotolerance in KG-1 cells following a priming dose of 45°C/5 min or 42°C/30 min. Cells were given a priming heat dose of 45°C/5 min or 42°C/30 min. After 4, 6, and 24 h of incubation at 37°C, cells were challenged at 45°C or 43°C and plated for colony formation in soft agar supplemented with 150 units of rGM-CSF.

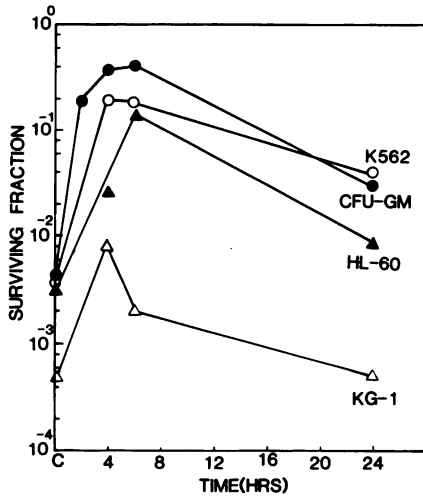
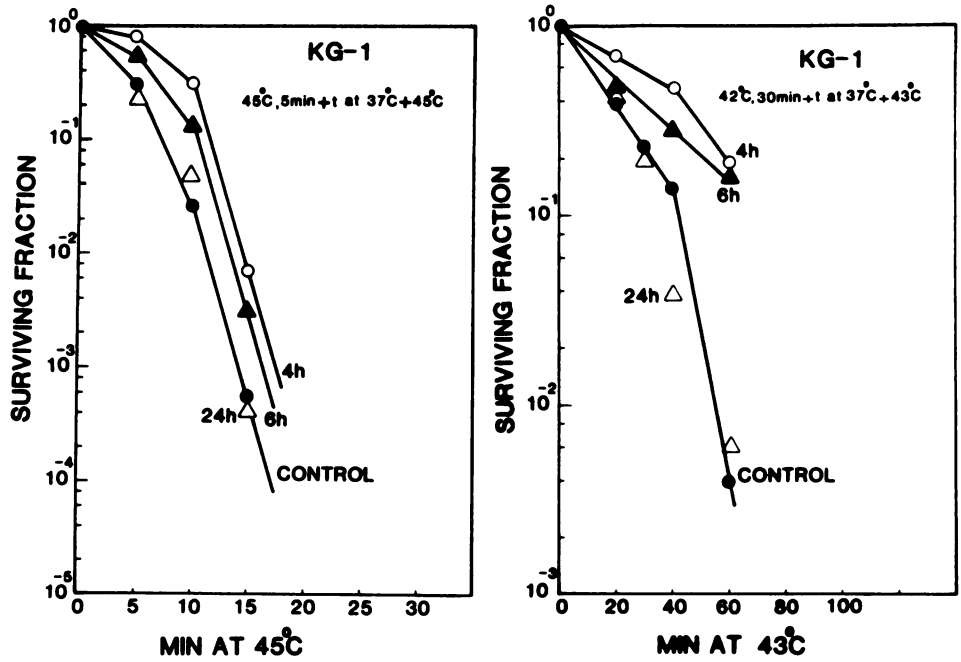
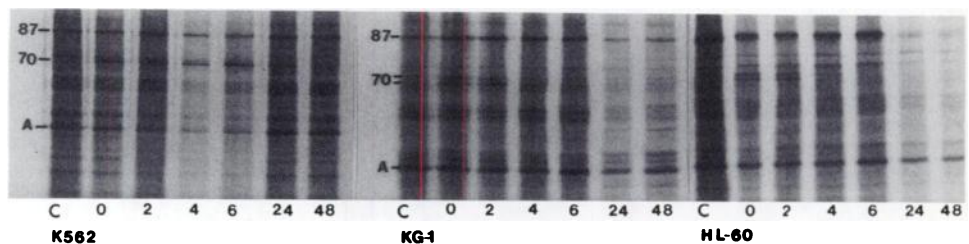


Fig. 6. Kinetics of thermotolerance induction and decay in leukemic cells and normal CFU-GM. K562 and normal CFU-GM received a priming heat dose of 45°C/10 min. KG-1 and HL-60 received a priming heat dose of 45°C/5 min. Cells were then incubated at 37°C for an increasing length of time before they received a challenging dose of heat. CFU-GM and K562 cells received 45°C/40 min, and KG-1 and HL-60 cells received a 45°C/15 min challenging dose. Results for normal CFU-GM were obtained from data presented in a previous publication (5).

tween some leukemias and normal bone marrow CFU-GM may be used to purge leukemic cells by fractionated heating *in vitro*. It is also possible to use fractionated WBH during bone marrow transplantation to destroy residual leukemic cells while taking advantage of systemic tolerance and tolerance to most other tissues (12, 13).

Heat-shock protein synthesis also differed in heat-sensitive and -resistant leukemic cells. In all cases, the M_r 87,000 HSP seems to be produced constitutively. However, the length of time where M_r 70,000 HSP synthesis continued following heat shock was shorter for heat-sensitive cell lines. Both KG-1 and HL-60 cells synthesized M_r 70,000 HSP for 2 to 4 h, while K562 cells synthesized M_r 70,000 HSP for over 24 h. Furthermore, 2-dimensional polyacrylamide gel electrophoresis analysis shows that K562 cells contain high levels of the inducible M_r 70,000 HSP in control cells (data not shown). The results presented here are similar to the previous reports (23, 24) that the synthesis of M_r 70,000 HSP following heat shock most closely correlates with the development and decay of thermotolerance. This lower amount of M_r 70,000 HSP produced in heat-sensitive leukemic cells may explain the lower amount of thermotolerance and a faster thermotolerance decay seen in heat-sensitive cell lines.

Fig. 7. Autoradiogram of sodium dodecyl sulfate-polyacrylamide gel of [³⁵S]methionine-labeled proteins of K-562, KG-1, and HL-60 cells after a priming heat dose of 45°C/10 min, 42°C/30 min, and 42°C/30 min, respectively. C, unheated control cells. 0, 2, 4, 6, 24, and 48 h, recovery time at 37°C following the priming heat dose. Molecular weights ($\times 10^{-3}$) are shown at the left.



ACKNOWLEDGMENTS

The author wishes to thank Saroj Kotecha for typing the manuscript.

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