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# A Possible Biochemical Basis for the Use of Hyperthermia in the Treatment of Cancer and Virus Infection

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There are numerous reports of tumors that have regressed or disapeared after artificial hyperthermia or a concurrent disease that caused high fever. Generally, local or whole body hyperthermia in the range of 41°–45°C has been involved. Overgaard (1978) has reviewed this literature. Current aspects of hyperthermia in the treatment of cancer has been the subject of several recent conferences (Converence on Hyperthermia in Cancer Treatment 1979; Thermal Characteristics of Tumors: Applications in Detection and Treatment 1980). A number of hypotheses have been advanced to account for these phenomena. Most of these have emphasized elevated blood glucose levels, hyperacidification, and breakdown of capillary circulation (Ardenne and Krüger 1979) or cytoplasmic damage resulting from changes in certain environmental factors such as hypoxia, increased acidity, and insufficient nutrition (Overgaard 1978).

Protein synthesis in intact cells and cell-free systems also is sensitive to inhibition by temperature in the range of 41°C to 45°C. The inhibition is reversible in intact cells and results in breakdown of polysomes without degradation of mRNA (Schochetman and Perry 1972; Goldstein and Penman 1973). The kinetics of protein synthesis inhibition caused by heating reticulocyte lysates to temperatures in the range of 41°C to 45°C are very similar to the novel inhibition curves observed with heme deficiency or double-stranded RNA (dsRNA) (Bonanou-Tzedaki et al. 1978a). Inhibition of protein synthesis is associated with a reduction in binding of Met-tRNA$_f$ to 40S ribosomal subunits (Bonanou-Tzedaki et al. 1978b). This binding reaction is mediated by peptide initiation factor 2 (eIF-2). We found that two components with inhibitory activity are activated by high pressure (Henderson and Hardesty 1978) or heat (Henderson et al. 1979). Both factors cause inhibition of protein synthesis when they are added to reticulocyte lysates. One is an acidic, heat-stable protein (HS) with an apparent molecular weight of about 30,000 daltons as estimated by gel filtration chromatography. HS has been purified more than 5,000-fold from reticulocyte lysates but has not been obtained in homogeneous form. An inhibition curve for a typical preparation of HS is shown in Fig. 1. We estimate that there is less than 30 μg of recoverable HS per 100 g of

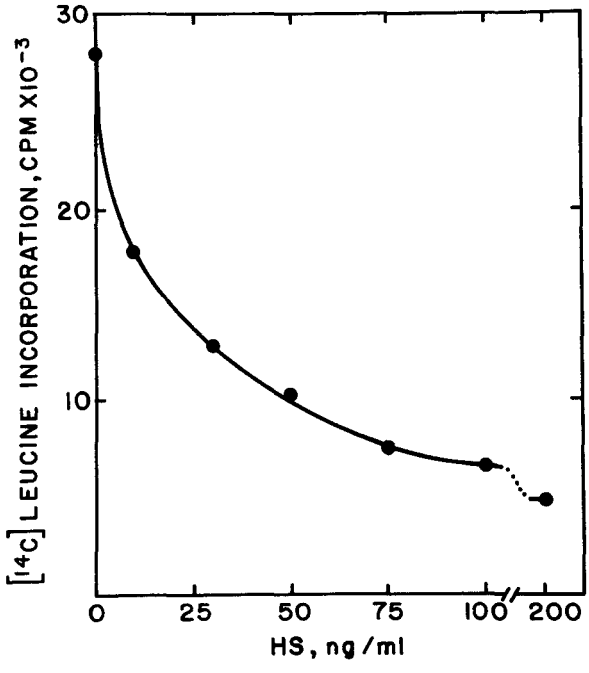


**Fig. 1** Heat stable (HS) inhibition of protein synthesis. HS$_i$, purified as described by Henderson et al. (1979), was activated by heating at 100° for 3 min. Aliquots were added to the reticulocyte lysate to give the indicated final concentration. Incorporation of [$^{14}$C]leucine (40 ci/mol) into protein was measured

protein in the postribosomal supernatant of rabbit reticulocytes. This corresponds to less than a few thousand molecules of HS per reticulocyte.

HS can be converted from an inactive form ($HS_i$) to an active species by heat or pressure. Activation of highly purified HS during a 5-min incubation at different temperatures is depicted in Fig. 2. Under these conditions appreciable activation is seen at 37°C. It is likely that HS is stabilized by other components in intact cells. HS has the unusual property of reverting to an inactive form if it is held at 0°C for 24–48 h. Table 1 shows the results of an experiment in which HS was cycled between the inactive and active form by repeated heating at 100°C for 3 min, followed by 24 h on ice. There is little or no loss of HS activity during this procedure. HS has little or no detectable inhibitory activity in the absence of the second factor, HL, which is a heat-labile protein of about 43,000 daltons. It appears that HS functions to inhibit protein synthesis by a catalytic, irreversible conversion of an inactive form of HL, $HL_i$, to an active species. The reaction is ATP-dependent but phosphorylation does not appear to be involved (Henderson et al., 1979).

The mechanism by which HS and HL inhibit protein synthesis appears to involve phosphorylation of the smallest subunit of eIF-2, eIF-2$\alpha$, by the specific protein kinase that is also activated in the absence of heme. This eIF-2$\alpha$ kinase appears to contain a peptide of about 100,000 daltons that is phosphorylated during activation by either HS-HL or by heme deficiency (Kramer et al. 1980). Antibodies against the eIF-2$\alpha$ kinase of the HCR system block inhibition by HS or HL. However, it should be emphasized that activation of this protein kinase by HS and HL occurs in the presence of heme. Thus HS and HL appear to form the distal elements of a cascade-type sequence of reactions that lead to inhibition of protein synthesis by phosphorylation of eIF-2$\alpha$. The relationship of the known components of the HS-HL and HCR systems that are involved in phosphorylation and dephosphorylation of eIF-2 is shown in Fig. 3.

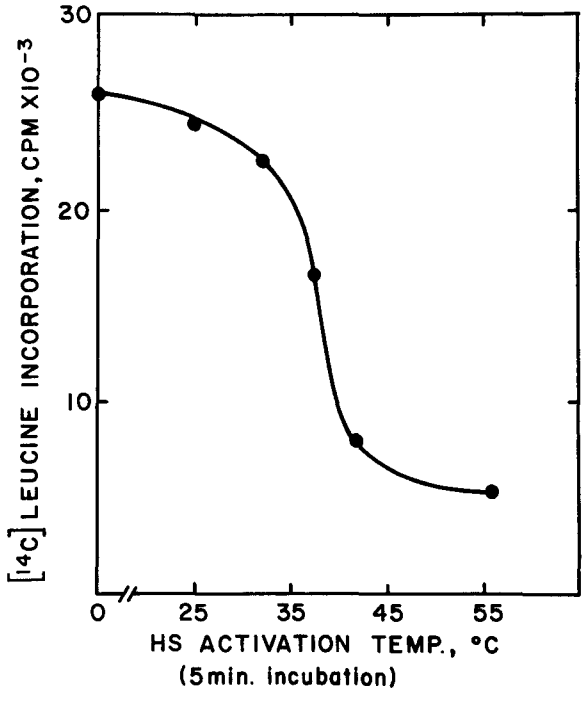


**Fig. 2.** Heat activation of HS. $HS_i$ was heated at the indicated temperature for 5 min, then inhibitory activity (100 μg HS protein/ml) was measured in the reticulocyte lysate system as described for Fig. 1

**Table 1.** Interconversion of $HS_i$ and HS by heating to 100°C followed by cold. A solution of purified HS similar to that used for the experiment of Fig. 1 was heated at 100°C for 3 min, then allowed to stand on ice for 24 h. The cycle of heating and standing in the cold was repeated as indicated. Aliquots containing 0.1 μg of HS protein were assayed in the reticulocyte lysate as described for Fig. 1

| Cycle number | Leucine incorporation into protein, cpm × $10^{-3}$ | |
|---|---|---|
| | Unheated | Heated |
| 1 | 28 | 8.9 |
| 2 | 29 | 9.3 |
| 3 | 28 | 9.1 |
| 4 | 27 | 8.6 |
| 5 | 28 | 10.1 |

Also shown in Fig. 3 is the relation of the components of the interferon-dsRNA system that appear to lead to phosphorylation of the same three sites of eIF-2$\alpha$ that are phosphorylated by the 100,000 dalton protein kinase of the HS-HL and HCR system (Samuel 1979). The protein kinase of the interferon-dsRNA system involves a 67,000 dalton peptide that also is phosphorylated during activation of the kinase. This occurs in the presence of dsRNA.

It is not established that the HS-HL system is activated during whole body or tissue hyperthermia. However, the biochemical studies indicated above considered with the effects of elevated temperature on protein synthesis apparatus of cells in culture make this seem likely. In turn, it appears that hyperthermia

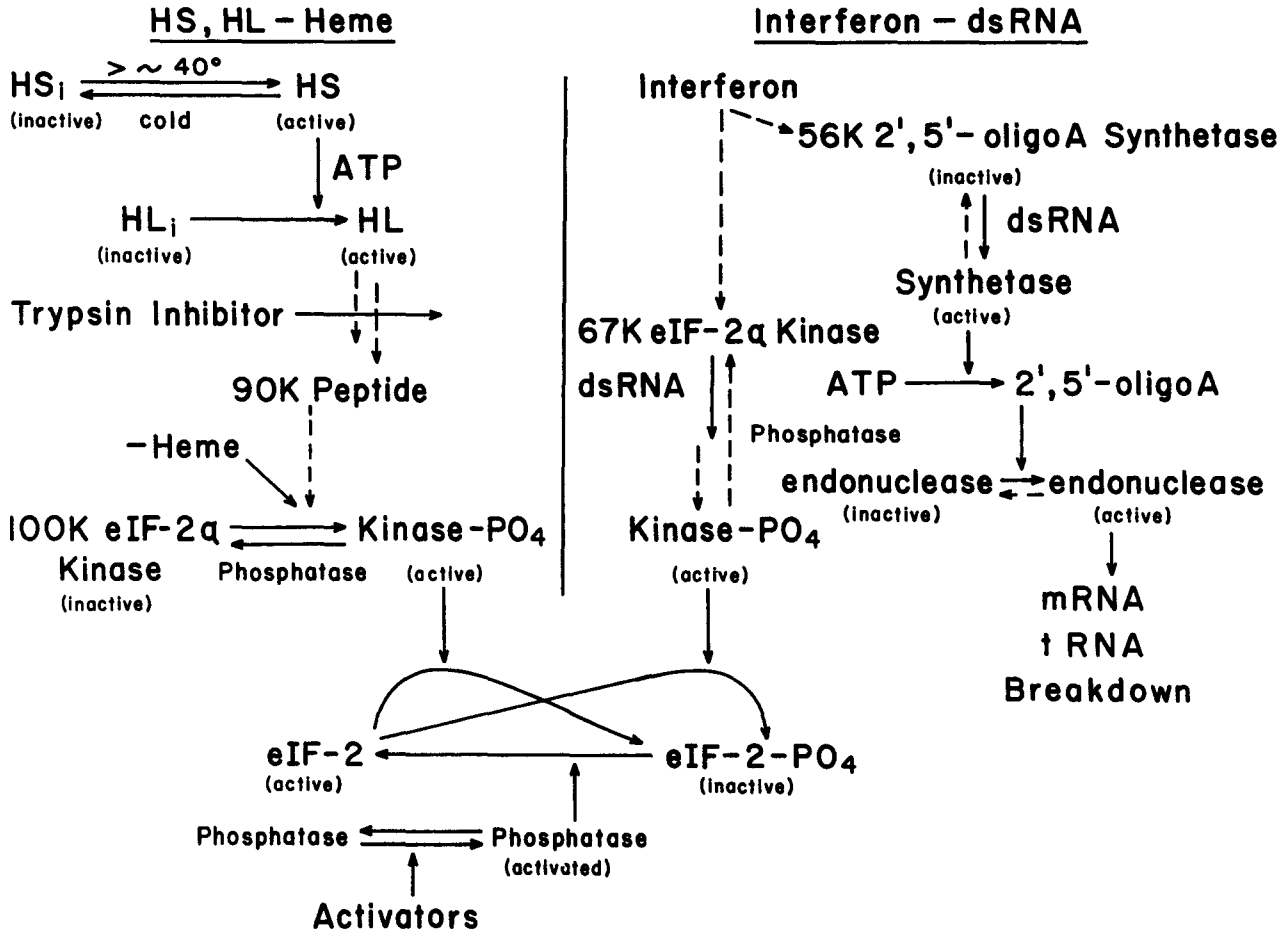


**Fig. 3.** Comparison of protein synthesis inhibition by HS-HL and by interferon-dsRNA. *Dashed lines* indicated unknown or uncertain relationship

and interferon may function by equivalent biochemical mechanisms to reduce the rate of peptide initiation. A change in this rate may not only slow the rate of cell growth but may also alter the relative proportion of proteins formed from different competing mRNA species (Kramer et al. 1980), thus altering the basic composition and physiology of the cell. Such a change brought on by activation of HS during high fever may constitute a basic defense mechanism for limiting translation of viral mRNA during viral infections and might be exploited for the treatment of cancer by hyperthermia.

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