If **1** is free **1**, F is free Zn^{2+}, B is bound Zn^{2+}, and K is the binding constant

$$K = [\mathbf{1} \cdot Zn]/[\mathbf{1}]\,[Zn] = B/[\mathbf{1}]\,F \quad (2)$$

Since the bound **1** is equal to the bound Zn, the total amount of **1** ($\mathbf{1}_t$) in the *cis* form becomes

$$\mathbf{1}_t = \mathbf{1} + B \quad (3)$$

Therefore

$$K = B/(\mathbf{1}_t - B)\,F \quad (4)$$

This equation can be rearranged to provide a form that is more convenient for plotting and extracting the value of K

$$B/F = K\,(\mathbf{1}_t - B) \quad (5)$$

From Fig. 3, a plot based on Eq. 5, we see that the binding of Zn to **1** follows Eq. 1 and we calculate that $K = 1.3 \times 10^5 M^{-1}$. A calculation of K from each of the individual points of the curve, with $\mathbf{1}_t = 0.6 \times 10^{-5} M$ as determined spectrophotometrically, yielded $K = 0.9 \times 10^5 \pm 0.1 \times 10^5 M^{-1}$. Therefore, our best estimate is $K = 1.1 \times 10^5 \pm 0.2 \times 10^5 M^{-1}$.

An examination of molecular models of the *cis* isomer reveals the possibility of intramolecular cooperativity between the two iminodiacetic acid groups, a phenomenon not possible in the *trans* isomer (Fig. 4). The planar *trans* form would require two molecules of **1**, and the binding of each molecule would be much weaker. These two factors probably account for the absence of Zn^{2+} binding by the *trans* isomer under the same conditions. Preliminary studies indicate that the ionization constants of *cis*-**1** and *trans*-**1** differ, which suggests that the binding of protons might also be amenable to photoregulation.

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Trauma-Induced Protein in Rat Tissues: A Physiological Role for a "Heat Shock" Protein?

Abstract. *Hyperthermic shock induces the synthesis of a novel protein (P_{71}) in many rat tissues in vivo. In incubated rat tissue slices P_{71} is the major protein synthesized even though it is undetectable in the tissues of a normal, unstressed rat. P_{71} is a "heat shock" protein, and it may be induced in vivo by stimuli other than hyperthermia. These results indicate that caution must be used in studies of protein synthesis in tissue explants, since the pattern of proteins synthesized by rat tissue slices is characteristic of stressed tissue.*

Freshly sliced rat tissues, including brain, thymus, heart, lung, spleen, liver, and kidney, rapidly synthesize a novel protein (P_{71}) which has a molecular weight of 71,000 and an acidic isoelectric point (*1*). This response was first observed in brain slices where the synthesis of P_{71} was traced to cells associated with the microvasculature (*2, 3*). P_{71} was not synthesized in unstressed brain in vivo, nor in brain slices during the first 30 minutes of incubation (*2*). In addition, it was shown that a new species of RNA was required for the synthesis of P_{71} (*2*). Although rat embryo cells in culture synthesize little or no P_{71}, these cells can be stimulated by canavanine, heat shock, or heavy metal ions to produce a protein similar, if not identical, to P_{71} (*1*). Similarly, one of the proteins synthesized in response to heat shock by *Drosophila* (*4*) and chicken embryo cultures (*5*) appears to be closely related to P_{71} (*6*). These studies showed that the induction of the heat shock proteins was accompanied by increased amounts of messenger RNA encoding these proteins.

Since P_{71} or proteins similar to P_{71} are induced by heat shock, canavanine, and heavy metal ions in various systems (in vitro), it has been suggested that this protein might be important for cell survival under conditions of environmental or physiological stress (*1, 7*). To examine the synthesis of P_{71} in vivo we used male Sprague-Dawley rats (*8*). First, we determined if P_{71} was synthesized or present in any tissues of either anesthetized or untreated rats. Second, we examined various tissues of rats stressed with hyperthermic shock for the synthesis of P_{71}. Proteins were separated and compared by a combination of isoelectric focusing (IEF) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Newly synthesized proteins were labeled in vivo by an intraperitoneal injection of 0.3 mCi (0.3 ml) of L-[^{35}S]methionine (*9*). At the end of a 90-minute labeling period, the rats were given 0.3 ml of Somnotol (*10*) and briefly perfused with saline via the left ventricle to remove as much blood as possible from the tissues (*11*). Various tissues (0.2 to 0.3 g) were removed and placed in 1.0 ml of lysis buffer for subsequent two-dimensional gel electrophoresis according to the method of O'Farrell (*12*). Samples (130 μl) were loaded on cylindrical columns (2 by 100 mm) for IEF in the first dimension. Focusing was done at 400 V overnight (15 to 18 hours) followed by 1 hour at 800 V. The IEF gels were equilibrated for 1 hour in SDS-sample buffer and the proteins were further separated by SDS-PAGE on 7.5 percent acrylamide slab gels (*13*). The gels were stained for proteins with Coomassie brilliant blue R and destained (*14*). Labeled proteins were visualized by autofluorography (*15*).

Figure 1A shows that P_{71} was the major protein synthesized by heart slices in vitro. The slices were prepared and incubated for 30 minutes in the absence of the labeled amino acid and then for 90 minutes in the presence of the label as previously reported (*1*). The two-dimensional gels were run as described above. The P_{71} was also synthesized at high rates in tissue slices of lung, brain, thy-

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mus, liver, spleen, and kidney (data not shown).

The synthesis and accumulation of P_{71} in vivo was undetectable in normal unstressed heart (Fig. 1B) and in other tissues including brain, thymus, lung, liver, spleen, pancreas, kidney, skeletal muscle, aorta, fat, salivary gland, and tongue (data not shown). The protein was also undetectable in rats that had been briefly anesthetized with ether for the injection of the radioactive label and in rats that were fully anesthetized with 0.1 ml of Somnotol for approximately 3 hours, the label being injected 90 minutes before the end of anesthetization.

In an attempt to stimulate the synthesis of P_{71} in vivo, we subjected male rats to brief hyperthermia. Hyperthermia was achieved by placing a rat, anesthetized with 0.1 ml of Somnotol, on a towel-covered warming plate set at 55°C. Rectal body temperature was raised and maintained between 42.0° and 42.5°C for 15 minutes. After the rats were cooled for 30 minutes to 37°C, L-[^{35}S]methionine was injected into the intraperitoneal cavity and the body temperature was maintained at 37°C for the 90-minute labeling period. Although the distribution of heart proteins, as determined by staining with Coomassie brilliant blue R, was unaffected by hyperthermic shock, the proteins synthesized by the heart (Fig. 1C) after hyperthermia were drastically altered. There was a striking increase in P_{71} synthesis with a concomitant decrease in the synthesis of most other proteins when compared to the normal unstressed heart. Similar observations were seen in the other tissues examined including brain, lung, thymus, liver, spleen, pancreas, kidney, skeletal muscle, aorta, fat, salivary glands, and tongue.

We do not know which cells synthesize P_{71} in vivo. The cells that synthesize P_{71} in brain slices were first located in the microvasculature (*2*, *16*) and then found to be localized in either the endothelial cell or pericyte (*17*). In rabbit brain, a 75,000-dalton protein, the synthesis of which was induced by hyperthermia, was found both in a microsomal fraction and a synaptic plasma membrane fraction (*18*). If this protein is related to rat P_{71}, then the induction of P_{71} synthesis by hyperthermia may occur in cells other than those related to the microvasculature. The present finding of the induction of P_{71} synthesis in many tissues in response to hyperthermic shock may indicate that either a ubiquitous cell type, such as the endothelial cell, or many different cell types (myocardial, neuroglial, epithelial, and smooth muscle cells, for example) can synthesize P_{71} in response to trauma.

The synthesis of P_{71} in vivo supports the contention that this "heat shock" protein has a physiological role. It is synthesized in all organs tested in vitro and in vivo in response to stress. In rabbits, an apparently similar protein with a molecular weight of 74,000 was induced in the brain by hyperthermia (*19*). Although we have also stimulated the synthesis of P_{71} in vivo by hyperthermia, heat shock may not be the physiologically relevant stimulus for P_{71} induction. At least three lines of evidence support this hypothesis. First, in one animal, ligation of the common carotid arteries for 30 minutes stimulated the synthesis of P_{71} in the brain, liver, and kidney, but not in the heart, lung, and spleen. In contrast to the tissue responses to hyperthermia, overall protein synthesis was not suppressed in this rat nor was P_{71} the major protein synthesized during the 90-minute labeling period. Second, heat shock was not involved in the preparation and incubation of tissue slices, yet the synthesis of P_{71} in slices was intense. Third, rat embryo cultures synthesize P_{71} in response to heavy metal ions or canavanine (*1*). We emphasize that organ slices do not reflect the synthesis of protein in vivo, but do reflect the response of tissue to various stressors. Tissue slices may prove useful in the elucidation of the stress response, although they must be used with caution by investigators studying "normal" cellular mechanisms.

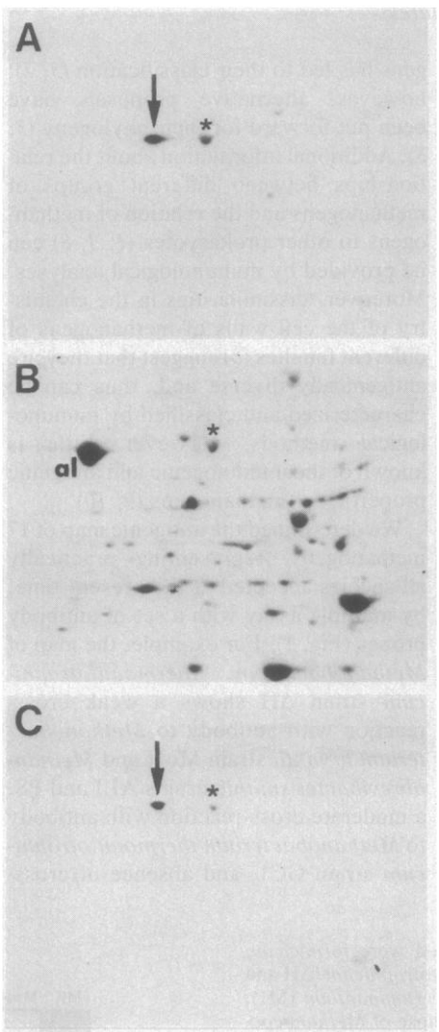

Fig. 1. Autofluorographs of radioactive proteins synthesized by (A) heart slices, (B) unstressed heart in vivo, and (C) heart in vivo after the animal was subjected to a 15-minute period of hyperthermic shock. The proteins were separated first by isoelectric focusing (horizontal dimension; acid *p*H is on the right) and second by SDS-PAGE (vertical dimension; low molecular weight on bottom). The amount of protein loaded on each gel was approximately equal. The synthesis of P_{73} (see asterisks) is approximately equal under each condition. P_{71}, indicated by arrows, becomes the major protein synthesized after either stress, but is undetectable between albumin (*al*) and P_{73} in the normal unstressed heart. (A) Heart slices were incubated for 90 minutes with [^{3}H]leucine after a 30-minute preincubation period. (B) Proteins were labeled for 90 minutes after an intraperitoneal injection of L-[^{35}S]methionine in a rat anesthetized with Somnotol. (C) Newly synthesized proteins were labeled with L-[^{35}S]methionine after a 15-minute period of hyperthermic shock (42.0° to 42.5°C) and a 30-minute cooling period. Body temperature was maintained at 37°C during the labeling period.

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