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# Heat shock inhibits and activates different protein degradation pathways and proteinase activities in *Neurospora crassa*

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*Abstract:* In *Neurospora crassa*, heat shock treatment inhibits proteolytic activity. ATP-independent proteinases were analysed after polyacrylamide gel electrophoresis using renaturing gelatine gels. Proteinases of 24, 29, and 130 kDa were shown to be inhibited by heat shock and were further characterized as to their properties. A major part of the heat shock-induced inhibition is probably due to suppression of de novo synthesis of proteinases as deduced from experiments with cycloheximide. During several hours of recovery from heat shock, the inhibition of overall protein degradation and ATP-independent proteinases is reversed. Azocasein assays as well as pulse–chase experiments further showed that ATP-dependent protein degradation is only slightly affected by heat shock. Two ATP-binding proteinases of about 60 and 160 kDa even show an increased activity after heat shock. The degradation rate of heat shock proteins is inhibited by heat shock treatment, indicating that they are degraded by ATP-independent proteinases. Western blot analysis of a ~ 40-kDa degradation product of HSP70 containing its amino terminal portion revealed a reduction in the amount of this peptide after heat shock.

*Key words:* Protein degradation; ATP-binding proteinase; Heat shock protein degradation; *Neurospora crassa*

## Introduction

Proteolysis is a major component of the cellular response to stress, particularly to heat shock [1,2]. In *Neurospora crassa*, the heat shock response has mainly been analysed at the level of heat shock gene expression [3–6]; however, not at the level of protein degradation.

Heat shock proteins (HSPs) are known to function as molecular chaperones [7] which rescue misfolded proteins or guide them to sites of degradation, for example to lysosomes or vacuoles, and to the multicatalytic proteinases (proteasomes) in the cytoplasm and nucleus [8]. Parts of the latter ATP-dependent degradation pathways were shown to be heat-inducible [2].

We analysed heat shock-induced changes of the general protein degradation rate and detected a considerable decrease after exposure to 42°C. We were especially interested in the adaptation behavior of protein degradation during

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longer exposures to increased temperature, a process which is also observed in the synthesis rates of proteins, including heat shock proteins [1].

Heat shock effects on single proteinases were assayed in renaturing gelatine gels, a method which allows analysis of particular properties of these enzymes [9]. The experiments revealed a strong inhibition of ATP-independent proteolytic activity by heat shock and also by treatment with a protein synthesis inhibitor, cycloheximide (CHX) indicating a synthesis block of these proteinases by both treatments. At least two ATP-binding proteinases, on the other hand, were shown to be transiently induced by heat shock.

HSPs are degraded by as yet not clearly defined pathways. Some of the HSPs are believed to exhibit proteinase activity themselves; HSP70, for example, as an autoproteinase [10]. Some other HSPs, such as HSP104 and HSP78, are homologous to the Clp family [11] known to associate with ATP-dependent proteinases or to act as chaperones. HSP70 degradation appeared to be slow and even slower after heat shock or continued exposure to elevated temperature as concluded, for example, from the lower amount of a ~ 40-kDa cleavage product after heat shock.

## Materials and Methods

*Strain and culture conditions*

We used the bdA strain (FGSC No. 1858) of *N. crassa*, a strain with particularly clear circadian conidiation pattern. For this reason this strain was analysed frequently in our laboratory. The other properties of the mutant are, however, very similar to the wild-type. *N. crassa* was cultured in Vogel's minimal medium [12] supplemented with 1 mM $CaCl_2$ and 2% sucrose at 25°C, starting with $10^6$ conidia $ml^{-1}$. Stock cultures were maintained on slants of Horowitz medium [13].

*Sample preparation*

For the overall degradation experiments and for proteinase activity measurement mycelia were harvested by filtration, broken with a precooled mortar and pestle after adding acid washed seasand and 0.1 M Tris · HCl buffer (pH 7.2) and then centrifuged (36000 × *g*, 1 h, 4°C). For isolation of ATP-binding proteinases, mycelia were broken using a Waring blender in buffer A (20 mM NaCl, 10 mM $MgCl_2$ in 20 mM Tris · HCl, pH 7.5) followed by sonication. The cell homogenate was then centrifuged (48000 × *g*, 40 min, 4°C). Protein concentration was determined according to [14] or [15].

*Proteolytic activity*

General proteolytic activity was measured by using the chromogenic substrate azocasein [16]. 25 μg total protein extract from control or treated cells were mixed with 1.6 ml substrate solution (0.2% azocasein in 10 mM Tris · HCl, pH 7.2) and incubated for 2 h at 37°C. After incubation, the non-degraded azocasein was precipitated by 10% trichloracetic acid (TCA), centrifuged (10000 × *g*, 15 min) and the extinction of degraded azocasein in the supernatant was determined at 366 nm. In order to quantitate the proteolytic activity of proteinases, a standard curve was determined with pronase (Serva).

For in vivo measurements of protein degradation, mycelia were incubated with L-[$^{35}$S]methionine for 3 h, then washed and resuspended in fresh medium containing 0.05% unlabelled methionine and incubated at different temperatures [17]. At defined times samples were taken and homogenized. Proteins were precipitated by TCA and the radioactivity (cpm) of the remaining L-[$^{35}$S]methionine-labelled proteins was determined in a scintillation counter [18], and used as a measure of the degradation rate in vivo.

In order to determine the activity of ATP-dependent proteinases in vitro L-[$^{35}$S]methionine-labelled proteins of *N. crassa* were used as substrate in a test system based on the methods of [19] and [20]. Labelled proteins from heat-treated (42°C, 2 h) cultures were isolated by gel filtration (NAP-10 columns, Pharmacia), dialysed, lyophilized and resuspended in Tris · HCl buffer (100 mM, pH 8). 100 μg of these labelled proteins (specific activity, 400 cpm (μg protein)$^{-1}$) were incubated at 35°C for 1 h in a system (500 μl) either able or unable to generate ATP (2 mM phosphocreatine, 0.16% creatine kinase, 5 mM ATP/$MgCl_2$, 25 μg protein extract from control

or heat-shocked cells in 100 mM Tris · HCl, pH 8, with or without 20 mM EDTA). Then proteins were precipitated with 10% TCA and centrifuged ($30\,000 \times g$, 30 min). The radioactivity (cpm) of the pellet was determined in a scintillation counter and the loss of radioactivity taken as a measure of proteolytic activity.

*ATP-binding proteinases*

Supernatants from the cell homogenates of untreated and treated mycelia were passed directly through a 10-ml ATP-agarose column (A2767, Sigma). After washing with buffer B (buffer A + 0.5 M NaCl), bound protein was eluted with buffer C (buffer A + 10 mM EDTA, without $MgCl_2$). The eluted fractions were then dialysed, lyophilized and analysed by means of gelatine polyacrylamide gel electrophoresis (G-PAGE).

*Gelatine polyacrylamide gel electrophoresis (G-PAGE)*

In order to determine the proteolytic activity of single proteinases, we applied the method of Heussen and Dowdle [9] with few modifications. Samples of the $36\,000 \times g$ supernatant were diluted in sample buffer (7% sucrose, 2.5% SDS), centrifuged ($10\,000 \times g$, 5 min) and pipetted into the gel slots. 1% gelatine as substrate of proteinases was co-polymerized in the 10% polyacrylamide gels. After separation of the proteins (50 V, 4°C) the gels were washed in NP40-buffer (2.5% NP40 in 50 mM Tris · HCl, pH 7.4) in order to remove SDS and allow renaturation of proteinases. Subsequently, the gels were incubated for 5 h at 35°C in buffer (5 mM $CaCl_2$ in 0.1 M glycine, pH 8.5), fixed, stained with Coomassie brilliant blue and destained. Bands which contain proteinases appear as white bands within a blue background. The enzyme-altered bands were quantitated densitometrically.

*Antibody preparation*

Rabbits were immunized with primary injections of 30 $\mu$g of dialysed and lyophilized HSP70 isolated from *N. crassa* [6] in 0.25 ml phosphate-buffered saline (PBS; pH 7.5) in a 1:1 mixture with complete Freund's adjuvant (Calbiochem) distributed to two subcutaneous sites on the back. Booster injections of 15 $\mu$g of HSP70 were given at 14-day intervals after primary injection in an identical manner except that incomplete Freund's adjuvant (Calbiochem) was used. For affinity-purification the antiserum was applied to columns with immobilized HSP70. Specific antibodies were eluted with 0.1 M glycine (pH 2.7), and neutralized and diluted (1:1000) in 0.2% Tween 20 containing PBS (PBST).

*Western blot analysis*

For Western blotting, the proteins were separated by one-dimensional 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; [21]) and transferred to a nitrocellulose membrane using a Trans Blot Cell (Bio-Rad) [22]. The blots were blocked with PBST for 1 h at 25°C. After removal of the blocking solution, primary antibody (affinity-purified polyclonal rabbit anti-HSP70 against the major *N. crassa* HSP70; [6]) diluted in PBST (1:1000), was added and left for 1 h at 25°C. Subsequently, the blots were washed with PBST. Secondary antibody (goat anti-rabbit IgG alkaline phosphatase conjugate, Bio-Rad) diluted in PBST (1:5000) was added and after 1 h at 25°C the blots were washed with PBST. The immuno-complex was detected by nitro blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (Boehringer, Mannheim, FRG).

*Protein sequence analysis*

Proteins were separated in one-dimensional 10% SDS-PAGE and transferred to polyvinylidene difluoride membrane. The N-terminal amino acid sequence of the ~ 40-kDa protein was then analysed by automated Edman degradation (Knauer, Berlin).

## Results

*Total proteolytic activity*

We analysed total proteolytic activity in vitro by using azocasein as the substrate. Mycelia were raised in growth medium for 24 h at 25°C, then transferred to Petri dishes with fresh growth

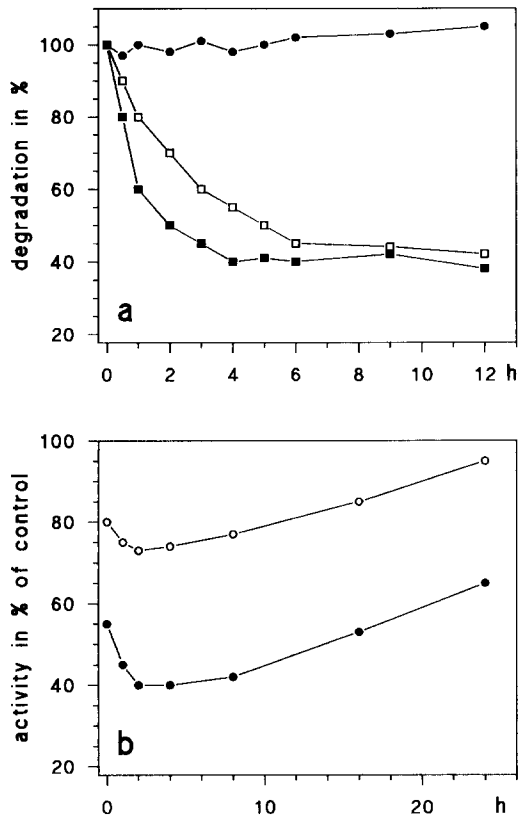


Fig. 1. Effect of different temperatures on proteolytic activity measured by azocasein digestion. (**a**) Effect of continued cold (4°C, □) and heat (42°C, ■) exposure of mycelia on the activity of proteinases in the homogenate (after 2 h incubation with azocasein at 37°C). ●, control culture at 25°C. Ordinate, digested azocasein in % of control; abscissa, time in h. (**b**) Recovery of protein degradation activity at 25°C after a 15-min (○) or a 30-min (●) heat shock at 42°C; ordinate as in a, abscissa: time after heat shock in h.

medium and incubated either at 25°C (control), 4°C or 42°C for 12 h. At regular intervals, samples were taken from the three cultures, and the proteolytic activity determined by means of the azocasein assay. The proteolytic activity measured before transfer to the different temperature conditions was taken as 100%. The results show an inhibition of proteolytic activity in both 4 and 42°C cultures (Fig. 1a). 6 h after transfer the proteolytic activity reached a steady state level of about 40 –45% of the control. This result demonstrates that low and high temperatures cause a considerable decrease in total proteolytic activity.

We followed the in vivo recovery of proteolytic activity at 25°C after 15 and 30 min exposure of the mycelia to 42°C by using the same azocasein test. The proteolytic activity decreased as a function of time of the heat exposure and initially continued to diminish after the transfer from 42°C to 25°C. A recovery of the proteolytic activity in the two cultures started 2–3 h after the transfer to 25°C (Fig. 1b). The culture exposed to 42°C for 15 min reached the control level after about 24 h of recovery. These experiments indicate a rather rapid break-down of the proteinases upon heat exposure and a slow recovery.

In order to examine the effects of heat shock on the degradation of cellular proteins, we labelled cellular proteins with L-[$^{35}$S]methionine and measured their degradation under different temperature conditions by means of pulse–chase experiments. After incubation of mycelia with L-[$^{35}$S]methionine for 3 h, the mycelia were washed and transferred to fresh medium with 0.05% unlabelled methionine and then exposed to different temperatures (20, 30 and 42°C) for 24 h. The radioactivity of acid-precipitable proteins at the start of the experiment was taken as 0% degradation. The decrease of radioactivity in the samples taken at defined times during the incubation was expressed in percent of the value at time 0. The decrease of radioactivity at 20 and 30°C shows an identical time course whereas the decrease at 42°C is slower, particularly evident after about 5 h (not shown). These experiments with cellular proteins thus confirm the results of the azocasein measurements.

In order to test whether the effect of heat shock on degradation differs in proteins with high and low turnover rates, we incubated mycelia with L-[$^{35}$S]methionine for short and long durations: proteins labelled for 30 min generally show higher turnover rates than those labelled for 12 h. The latter group also includes proteins of higher turnover rates, which, however, affect the long-term kinetics only to a minor extent. The experiments show that the briefly labelled proteins were in fact degraded faster. Their degradation is less inhibited by 42°C compared to the proteins labelled for 12 h, whose degradation rate at 42°C is inhibited by a factor of 2–3 (not shown). This

result may be due to the fact that short-lived proteins are degraded mainly by ubiquitination and ATP-dependent pathways which are less suppressed or even induced by higher temperatures [8].

A possible interpretation of the inhibitory effect of heat shock on protein degradation is to assume that 42°C inhibits de novo synthesis of proteinases, especially ATP-independent proteinases, as is true for most proteins. Support for this assumption comes from experiments in which we tested the effect of the protein synthesis inhibitor cycloheximide (CHX) on protein degradation (Fig. 2): an almost complete inhibition of the degradation of cellular proteins is observed during continuous incubation with 1.6 $\mu$g ml$^{-1}$ CHX. Another substance which inhibits protein synthesis very strongly is 2,4-dinitrophenol (DNP), a substance which also lowers the intracellular pH and uncouples mitochondrial ATP synthesis. 1 mM DNP inhibits the protein degradation rate also very strongly (Fig. 2). Furthermore, we tested the effect of ethanol which in *N. crassa* inhibits protein synthesis by about 30% at a concentration of 0.8 M (Meyer et al., submitted). Ethanol also inhibits protein degradation (Fig. 2).

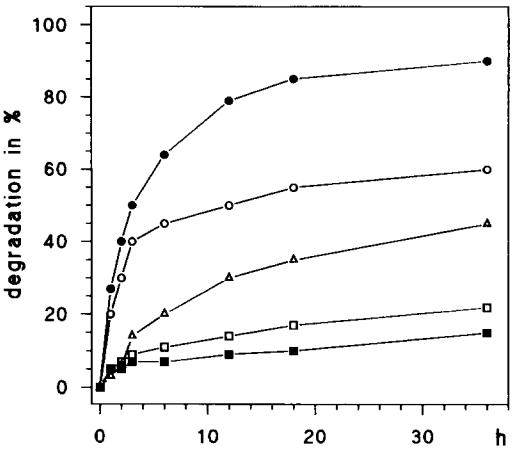


Fig. 2. Effect of different inhibitors on proteolytic activity determined in vivo by pulse-chase experiments. ●, control at 25°C; ○, 100 $\mu$M HMB; △, 0.8 M ethanol; □, 1 mM DNP; ■, 1.6 $\mu$g ml$^{-1}$ CHX. Ordinate, Loss of L-$^{35}$S-label after a 2-h incubation with L-[$^{35}$S]methionine; abscissa, time in h.

In order to estimate the proportion by which serine and cysteine proteinases participate in proteolytic activity, we incubated mycelia with various artificial proteinase inhibitors (100 $\mu$M phenylmethylsulfonylfluoride, PMSF; 100 $\mu$M *N*-tosyl-L-phenylalaninechloromethyl-ketone, TPCK and

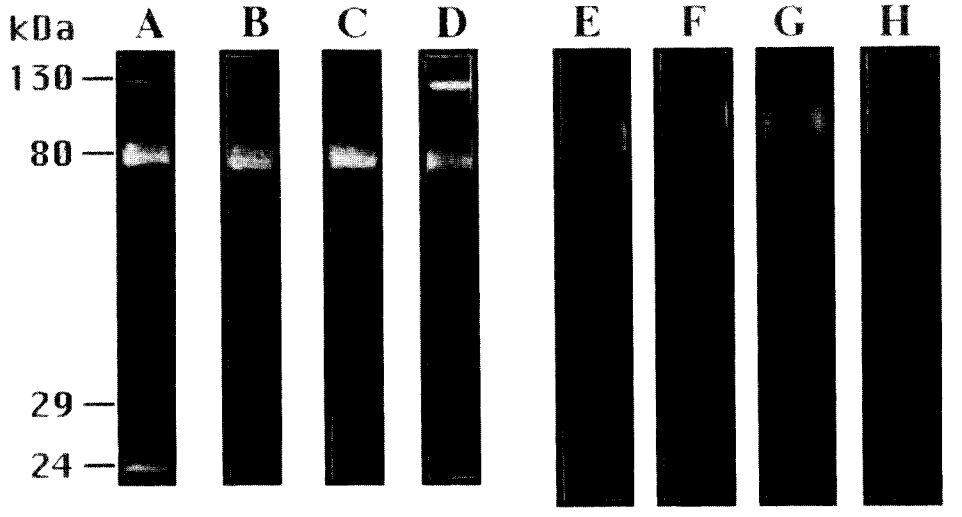


Fig. 3. Effects of different temperatures and inhibitors on proteinase activity as determined by G-PAGE. Control at 25°C (A); after exposure of mycelia to 42°C (B) or 4°C (C) for 2 h; after incubation of the gels without $Ca^{2+}$ (D); with 100 $\mu$M HMB (E); with 100 $\mu$M PMSF (F); and after incubation of mycelia with 1 mM DNP (G); or with 1.6 $\mu$g ml$^{-1}$ CHX (H) for 2 h.

100 $\mu$M hydroxymercuribenzoate, HMB). Among these inhibitors, HMB showed the strongest inhibition (Fig. 2), whereas PMSF was less and TPCK least effective (not shown).

*Single proteinases*

In order to characterize some of the ATP-independent proteinases affected by heat shock we used gelatine polyacrylamide gel electrophoresis (G-PAGE; [9]) which allows the analysis of their molecular mass, pH optimum, sensitivity to inhibitors etc. Cytoplasmic extracts of mycelia kept at 25°C (control) show 4 main bands of 24, 29, 80 and 130 kDa (Fig. 3A). The 80-kDa band consists of at least three bands of slightly different molecular masses. A 2-h incubation of mycelia at 42°C abolishes the activity of the 24-, 29- and 130-kDa proteinases (Fig. 3B). Kinetic studies show that the 29-kDa proteinase is completely inhibited after 45 min and the 24-kDa proteinase after 50 min exposure to heat shock (not shown). In mycelia exposed to 42°C for 2 h and then shifted to 25°C the activity of the 2 proteinases reappeared 1 h after the temperature shift and reached control levels again after about 5 h. Exposure to 4°C for 2 h also abolished the activity of the same proteinases (Fig. 3C).

Incubation of the gels with 50 $\mu$M EDTA or with 100 $\mu$M HMB prevented the activity of the 24- and 29-kDa proteinases (Fig. 3D,E), whereas 100 $\mu$M PMSF inhibited the 80- and 130-kDa proteinases (Fig. 3F). These results indicate that the 80- and 130-kDa bands represent serine proteinases and the 24- and 29-kDa bands cysteine proteinases which require metal ions ($Ca^{2+}$, $Mg^{2+}$) for their activity. Since there was no ATP in the incubation medium, all proteinases are ATP-independent.

In vivo incubation of mycelia for 2 h with 1 mM 2,4-DNP or with 1.6 $\mu$g $ml^{-1}$ CHX affected all proteinases (Fig. 3G,H) suggesting that the activity of these enzymes rely on continuing de novo synthesis.

*ATP-dependent protein degradation*

Major pathways of protein degradation involve ATP-dependent ubiquitination and ATP-dependent degradation [8]. In order to estimate the heat shock effect on ATP-dependent degradation we used cytoplasmic extracts of control and heat-shocked mycelia. Lower molecular mass proteins as well as ATP and other compounds were eliminated from the extracts by gel chromatography. Equal concentrations of these proteinase-containing extracts were then incubated with L-[$^{35}$S]methionine-labelled proteins from heat-treated *N. crassa* cells as substrate in a system able or unable to generate ATP. The results showed that the proteolytic activity without ATP was severely inhibited by the heat shock treatment (29% of the control), whereas the ATP-dependent proteolysis is apparently not affected.

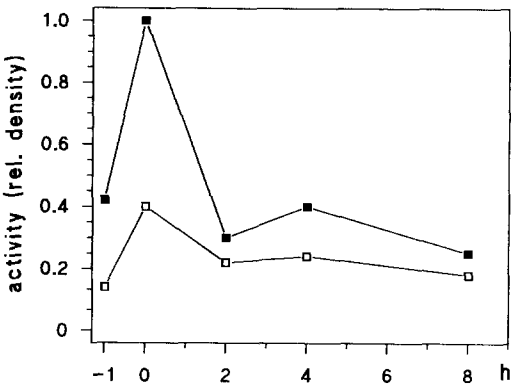


Fig. 4. Effects of heat shock (45°C, 1 h) on the activity of 60 kDa (□) and 160 kDa (■) ATP-binding proteinases as determined by G-PAGE. Ordinate, proteinase activity was measured by densitometry and are expressed relative to the value 1 assigned to the most intense band in the gel. Abcissa, control at 25°C (−1 h), directly after heat shock (0 h), and after 2, 4 and 8 h recovery from heat shock at 25°C.

Isolation of ATP-binding proteinases by means of affinity chromatography and subsequent analysis on G-PAGE revealed two proteinases of about 60 and 160 kDa which were induced (60 kDa) or enhanced (160 kDa) in their activity after 1 h heat shock (45°C). Their activity returned to normal levels after about 4 h of recovery at 25°C (Fig. 4).

*Degradation of HSP70*

In order to analyse the degradation of HSP70 after heat shock and during continued exposure to 42°C we analysed changes in the amount of a

degradation product of HSP70. HSP70 is cleaved into a ~ 40- and a ~ 27–30-kDa peptide [10] in an initial degradation step. The ~ 40-kDa peptide also reacts with the polyclonal antibody and can be determined on Western blots (Fig. 5a). In order to unambiguously identify this peptide as a degradation product of HSP70, we sequenced the amino terminus. This sequence (APAVGIDLGTTY) is identical with that of the amino terminal end of the major HSP70 [6].

The results of the recovery experiments show (Fig. 5b) that the amount of the ~ 40-kDa peptide decreases to about 50% of the control 30 min after a 1-h heat shock (45°C) and then recovers to values which approach control levels 2–6 h after heat shock. The HSP70 level shows a drastic increase after a 1-h heat shock followed by a decrease. When *N. crassa* was exposed continuously to high temperature (42°C), the early decrease of HSP70 is slowed down, and the recovery in the amount of the ~ 40-kDa peptide is suppressed (Fig. 5c).

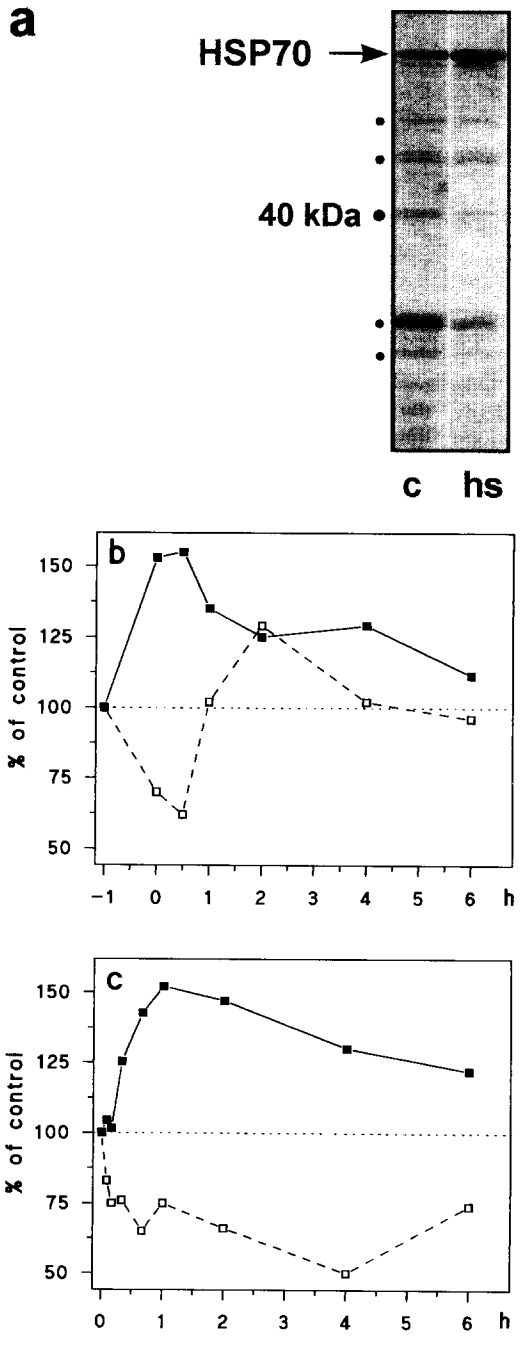


Fig. 5. Changes in the amount of HSP70 and a HSP70 degradation product ( ~ 40-kDa protein) after heat shock and during continued exposure to elevated temperature. (**a**) Western blot with a polyclonal antibody against HSP70. c, control; hs, after a 1-h heat shock. Points indicate possible degradation products of HSP70, the ~ 40-kDa fragment contains the amino terminal fraction of HSP70. (**b**) Amount of HSP70 (■) and its degradation product ( ~ 40-kDa protein, □) after 1 h heat shock (45°C) during recovery at 25°C. (**c**) Amount of HSP70 (■) and its degradation product (□) during continued exposure to 42°C. Ordinate, amount in % of control; abscissa, time after heat shock (**b**) or beginning of heat treatment (**c**) in h.

## Discussion

The observed inhibition of total proteolytic activity as well as of the activity of proteinases has also been described in other organisms [1]. In the basidiomycete *Schizophyllum commune*, heat shock inhibited several proteinases as determined by G-PAGE [23]. Similar results were obtained in HeLa cells [24]. The inhibition of total proteolytic activity in *N. crassa* is attributable mainly to ATP-independent cytoplasmic and lysosomal proteinases whose activity may depend on de novo synthesis, as concluded from the inhibitory effects of cycloheximide. Cycloheximide was shown to

inhibit protein degradation also in mammalian cells [25,26]. The ATP-independent proteinases seem to degrade long-lived proteins more than short-lived proteins. Their recovery from heat shock treatment needed considerable time, in the order of 12 h to more than 24 h in our experiments depending on the previous length of time of exposure to 42°C.

ATP-dependent protein degradation consists of the ubiquitin-dependent pathway responsible for about 90% of the short-lived proteins in a mamma carcinoma cell line [20] and large cytoplasmic complexes (proteasomes) involved in the degradation of ubiquitinated proteins [8]. In yeast, four of the eight proteins involved in ubiquitin conjugation of proteins during a stress response, particularly the activity of the UBC4 and UBC5 genes, were shown to be heat-inducible [27]. Short-lived and malfolded proteins are the main subjects of ubiquitin conjugation. Ubiquitinated proteins are degraded in a large multifunctional proteinase complex, the 26S-proteasome, but were also found in the vacuole [28]. The degradation of some proteins which do not become conjugated to ubiquitin is also ATP-dependent [29].

The subunits of the high molecular mass (1500 kDa) proteasome complexes have molecular masses of 20–30, 40–110 and $>110$ kDa [8], some of which may correspond to the ATP-binding proteinases which we detected after G-PAGE. The proteolytic activity of the separated monomers, however, is unknown and may be confined to cleavage of short peptides [2]. In our experiments, the kinetics of the heat shock-induced activity of ATP-binding proteinases would support the assumption of de novo synthesis of these proteinases and their classification as HSPs.

HSPs play a significant role in protein degradation either by facilitating the import of proteins containing a recognition sequence (KFERQ or similar amino acids) into lysosomes (HSP73; [30]) or by associating with proteinases in proteasomes [2,8]. Both functions enhance protein degradation and are believed to rescue cells from possibly toxic effects of denatured and aggregated proteins. A heat shock-induced change in the degradation rates of HSPs themselves is an interesting problem in this context because of its adaptive significance. HSP70 shows two closely associated chymotrypsin and trypsin cleavage sites that yield stable 44–45 kDa, N-terminal fragments containing the ATP-binding site [31,32]. Mitchell et al. [10] proposed an autoproteolytic activity of HSP70, as deduced from HSP70 fragmentation during electrophoresis. One of these fragments described by the authors showed a molecular mass of 43 kDa, which seems to correspond to the about 40-kDa fragment in our experiments.

From the lower amount of this fragment after heat shock a slower degradation rate of HSP70 may be deduced. This would contribute to the prolonged maintenance of higher HSP70 levels during stress in order to cope with the increased requirements for HSP70 and would correspond to the increased stability of the HSmRNA [33].

The different analytical approaches led to different recovery kinetics of the inhibitory as well as of the activating effects: the HSP70 degradation fragment already reached normal levels 2 h after heat shock. The suppressed activity of the ATP-independent proteinases needed about 2 h (130 kDa) or 5 h (24, 29 kDa) for complete recovery, whereas the total proteolytic activity needed more than 20 h to recover. Furthermore, the induced activity of ATP-binding proteinases (60 and 160 kDa) returned to control levels after about 4 h. These kinetic differences may be due to different mechanisms affected by the heat shock treatment. Changes in the synthesis and degradation of proteinases may result in a slower recovery than an unfolding and subsequent refolding or any covalent changes of the enzymes.

During longer exposure to higher temperatures, protein degradation adapts completely only at 30°C, whereas at 42°C a lower level of protein degradation (mainly of long-lived proteins) is maintained, as deduced from the experimental approaches we used. This adaptational response of the overall protein degradation is similar to the adaptational response of protein synthesis to different temperatures (Mohsenzadeh, unpublished): there is complete adaptation to 30°C but a permanent lower synthesis level at 42°C. The biological reason for such a parallel change in the synthesis and degradation rates may rest on the maintenance of relative constant protein levels.

This may be true, for example, in the maintenance of actin or HSP levels [1], and may be of particular importance in the temperature adaptation of the period length of circadian rhythms [34] which includes synthesis and degradation of an oscillatory protein. A parallel change in the synthesis and degradation of proteins suggests a common link between the control mechanisms already proposed [35].

From our experiments, we conclude that a large proportion of proteins, mainly the long-lived proteins, including HSP70, is degraded by ATP-independent proteinases whose activity is inhibited by heat shock. The activity of these proteinases seems to depend at least partly on de novo synthesis which thus constitutes a link between protein synthesis and degradation. ATP-binding proteinases were shown to be activated by heat shock, which is consistent with the finding that the ATP-dependent degradation of proteins and the degradation of short-lived proteins is little affected or even enhanced by heat shock.

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