Plant Physiol. (1990) 92, 740–746
0032-0889/90/92/0740/07/$01.00/0

Received for publication August 18, 1989
and in revised form October 30, 1989

Ubiquitin Pool Modulation and Protein Degradation in Wheat Roots during High Temperature Stress[1]

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ABSTRACT

Ubiquitin, a key component in an ATP-dependent proteolytic pathway, participates in the response of various eucaryotic organisms to high temperature stress. Our objective was to determine if ubiquitin serves a similar capacity for metabolizing altered proteins in higher plants during stress. Degradation of total proteins was measured, and ubiquitin pools (free *versus* conjugated) were extracted with an improved protocol from wheat (*Triticum aestivum* L. cv Len) roots treated at 22, 27, 32, 37, and 42°C for 1 hour and assayed by western blots and radioimmunoassays. Heat-shock protein synthesis was detected by *in vivo* labeling and autoradiography. Mean half-life of total root proteins decreased from 51 hours at 22°C to 23 hours at 40°C. Ubiquitin pools were extracted better and proteolysis was slowed more by the improved protocol than by a conventional procedure for plant proteins. Amounts of high molecular mass conjugates were elevated and levels of low molecular mass conjugates and free ubiquitin were depressed when roots were treated at 37 or 42°C than at lower temperatures; the same high temperatures also induced synthesis of heat-shock proteins. We concluded that high temperatures increase breakdown of root proteins, which are degraded via the ubiquitin proteolytic pathway. A conjugate with an apparent molecular mass of 23 kilodaltons was tentatively identified as an ubiquitinated histone.

Ubiquitin is a small protein (8.5 kD) that occurs in all eucaryotes and differs in only three amino acid substitutions between mammalian and plant forms (34). It is a key component of an ATP-dependent cytosolic proteolytic pathway that is responsible for degrading proteins with short half-lives (20, 23, 32).

The ubiquitin proteolytic pathway was originally characterized in immature red blood cells (20); however, a nearly identical pathway occurs in plants (19, 32, 33). The pathway is initiated by conjugation of ubiquitin to proteins that are to be degraded. Other ubiquitins conjugate to the first one, forming a polyubiquitin chain (7). These polyubiquitin-protein conjugates then are degraded by an ATP- and ubiquitin-dependent multicomponent proteolytic complex that releases small peptides and intact ubiquitin (13, 22).

Ubiquitin has been implicated in high temperature stress responses of mamalian cells (27), avian cells (3, 4), yeast (10), green algae (30), and the flowering plant, *Arabidopsis* (6). This involvement has been demonstrated by several different methods, including differential expression of ubiquitin genes during heat shock (4, 6, 12), sensitivity of mutants with altered ubiquitin metabolism to high temperature stress (10), and changes in ubiquitin pools (free *versus* conjugated forms) during heat shock (3, 27, 30). Intracellular concentrations of high molecular mass ubiquitin conjugates and proteins degraded via the ubiquitin proteolytic pathway increased in heat-shocked mammalian cells (27), and similar increases in high molecular mass ubiquitin conjugates occurred in heat-shocked avian cells (3) and green algae (30).

Involvement of ubiquitin in protein degradation (20, 32, 33) and activation of genes for its synthesis by heat shock (4, 6, 12) suggest a role for this pathway in responses of higher plants to elevated temperature. Our objectives were to quantify changes in free and conjugated ubiquitin in relation to protein turnover and induction of heat shock proteins during high temperature stress. Modification of a method used previously for extracting ubiquitin pools from cultures and single cells is described for extracting ubiquitin pools from plant tissue.

MATERIALS AND METHODS

Plant Materials

Wheat (*Triticum aestivum* L. cv Len) roots were used because high root temperature accelerates senescence of the entire plant (24), and their HSP[3] have been described (25). Seeds imbibed on moist filter paper until they germinated, usually 48 to 72 h, and seedlings were transplanted and grown in 13 × 100-mm test tubes having two plants per tube. The roots were supplied with 0.25-strength nutrient solution (21), which was continuously aerated. Growing conditions were constant 22°C, 16-h light period with PAR (400–700 nm) intensity of 225 μE $m^{-2}s^{-1}$, and eight-h dark period. Seedlings were used when they were 10 d old.

Protein Degradation Rates

Mean rates of total protein degradation were measured in roots labeled with ^3H_2O (50 μCi/mL) in 0.25-strength nutrient solution (21) for 48 h and chased at 22°C for 24 h before

[1] Contribution 89-528-J of the Kansas Agricultural Experiment Station.

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[3] Abbreviations: HSP, heat shock proteins; TBS, tris-buffer saline; uH2A, ubiquitinated histone 2A.

initiation of temperature treatments of 22, 32, and 40°C. During the labeling and the chase, the plants received a 16-h light regime (90 μE m^{-2} s^{-1}) and continuous aeration of the nutrient medium. After initiation of treatment, roots were periodically sampled over a 24-h period for measuring turnover rates by the method of Dungey and Davies (9). Total radioactivity associated with protein per mg of fresh weight of tissue was determined by liquid scintillation counting.

Temperature Treatments for Ubiquitin Responses

Temperature treatments were imposed on seedling roots by immersing the test tubes containing the plants in a circulating water bath set at 22, 27, 32, 37, or 42°C for one h. PAR of 65 μE m^{-2} s^{-1} and continuous aeration were supplied during treatment. Cadmium treatment, as an alternative method of inducing stress, was imposed by adding 250 μM Cd nitrate or Cd acetate for a period of 12 h to the nutrient solution of plants grown at 22°C.

Extraction of Ubiquitin Pools

Two methods, protocol A, a modified procedure for single-cell organisms, and protocol B, a conventional procedure for extracting protein from plants, were evaluated. The selected method, protocol A, was followed for extracting protein from the root tissue for measuring ubiquitin pools in all experiments except those in which extraction procedures were compared. For protocol A, roots of seedlings treated with high temperature or Cd were ground to an extremely fine powder in liquid nitrogen in a precooled mortar and pestle. The frozen root powder was transferred to a precooled microfuge tube, hot (95°C) extraction medium (50 mM Tris [pH 6.8], 4% [w/v]SDS, 10% [v/v] mercaptoethanol) was quickly added to the tissue (100 μL/50 mg tissue), and the suspension was mixed. The preparation then was heated at 95°C for 20 min with intermittent mixing at 5-min intervals. After cooling to room temperature, the mixture was centrifuged at 13,000*g* for 15 min. Aliquots of the supernatant were stored at −80°C.

In protocol B, roots were ground in liquid nitrogen, the powder was transferred to microfuge tubes, and extraction medium (50 mM Tris-HCl [pH 6.8], 5 mM iodoacetate, 20 μM leupeptin, 20 μM pepstatin, 1 mM EDTA, 2 mM PMSF, 1 mM *N*-tosyl-L-lysine chloromethyl ketone, 1% [w/v] SDS) at room temperature was added (100 μL/50 mg tissue). The slurry was vigorously mixed for 2 or 5 min before being centrifuged at 13,000*g* for 15 min. Equal volumes of the supernatant and SDS sample buffer (50 mM Tris-HCl [pH 6.8], 4% [w/v] SDS, 10% [v/v] mercaptoethanol) were mixed, and the resulting solution was heated at 95°C for 20 min. Aliquots of the extracts were stored at −80°C.

Because mercaptoethanol in the samples prevented standard protein analysis, the protein was quantitatively precipitated from solution with −20°C acetone, washed with cold acetone a second time, dried, and dissolved in 2% SDS. Protein concentration was determined in duplicate samples with the bicinchoninic acid protein assay reagent (31) using BSA in 2% SDS (w/v) as a standard.

Western Blots

Twenty μg of protein from each sample was separated by SDS-PAGE with a 10 to 17.5% linear gradient resolving gel and the buffer system of Fling and Gregerson (11). Proteins were transferred from the gel to 0.2-μm nitrocellulose with a polyblot apparatus (American Bionetics, Inc.). The blots were analyzed using a polyclonal, monospecific antibody made with bovine ubiquitin by the procedure of Haas and Bright (17). Antibodies bound to the blot were detected with ^{125}I-protein A and autoradiography.

Quantification of Ubiquitin Pools

Free ubiquitin in the root samples was quantified by radioimmunoassay with antiserum to free ubiquitin (18). The antiserum was made with bovine ubiquitin.

Relative changes in the quantity of conjugated ubiquitin were measured with a solid phase immunoassay (17). The root samples were initially diluted to 0.05-times their original concentration with TBS (50 mM Tris-HCl [pH 7.5], 150 mM NaCl), and additional dilutions were made with TBS containing 0.01% (w/v) SDS. The samples (60 or 100 ng protein) were applied to nitrocellulose with a slot blot apparatus (Schleicher and Schuell, Inc.). Immunochemical staining of the blot was by the method of Haas and Bright (17), using a polyclonal, monospecific antibody (provided graciously by Dr. A. L. Haas, Medical College of Wisconsin) with specificity toward the conjugated form of ubiquitin. Samples of free ubiquitin were included on each blot to ensure that binding to this form was negligible. A laser densitometer was used to quantify each slot on the autoradiogram.

Histone Extraction and Immunoblots

Histones were extracted from calf thymus by a procedure of Goldknopf *et al.* (15). The proportion of uH2A to the total histones was estimated by SDS-PAGE and densitometry. The uH2A band was verified by Western blotting and analysis with ubiquitin antibody. The preparation containing a known amount of ubiquitinated histone was intended to be the standard of conjugated ubiquitin in the radioimmunoassay. Difficulty was encountered with this approach, however, and only relative values are reported. The histone preparation also was used to compare the migration of uH2A with an unknown ubiquitin conjugate of similar mol wt in the root samples on radioimmunoblots.

Antibodies and protein A were desorbed from radioimmunoblots by incubation in 2% (w/v) SDS, 100 mM 2-mercaptoethanol, 50 mM tris-HCl (pH 7.5), and 150 mM NaCl at 60°C for 30 min. After several washes in TBS, the plots were reprobed with a monoclonal antibody with specificity toward mammalian histones. Binding of the histone antibody was visualized by immunochemical staining with anti-mouse-IgG antibodies linked to alkaline phosphatase.

Induction of HSP

Seedlings were grown as described above except that the nutrient solution was replaced with one with no nitrogen

sources one day before labeling to enhance incorporation. Ten-d-old seedling roots were subjected to a 3-h temperature treatment (22, 27, 32, 37, or 42°C) in a circulating water bath. The nutrient solution was replaced with an incubation buffer (1 mM potassium phosphate [pH 6.0]; 1% [w/v] sucrose; 20 μCi/mL L-[4,5-^{3}H]leucine) at the beginning of the temperature treatments. During treatment, the plants received 65 μE m^{-2} s^{-1} PAR and continuous aeration. Other seedlings held at 22°C received a 12-h Cd treatment, in which the roots were placed in a N-free nutrient solution containing 250 μM Cd acetate for the first 9 h. The nutrient solution was then replaced with incubation buffer containing the radiolabel and 250 μM Cd acetate for the last 3 h of Cd treatment.

Protein in roots was extracted by protocol A as described above. Total label incorporation into protein was determined by precipitating protein with 10% TCA and collecting the protein by filtration through a nitrocellulose membrane. Activity associated with the protein was determined by liquid scintillation counting.

Label incorporation into individual proteins was ascertained by SDS-PAGE and fluorography. Equivalent amounts of radioactivity for each sample were loaded on the gel, and proteins were separated by the SDS-PAGE system described above. Fluorography was with ENHANCE (Du-Pont-NEN) and Kodak XAR film.

Experimental Designs and Data Analyses

Mean half-life of protein at each temperature treatment was determined by linear regression of log % time zero (log [(dpm per mg tissue/dpm per mg tissue at time zero) × 100]) *versus* time of treatment.

Experiments to quantify free and conjugated ubiquitin with radioimmunoblots of root samples were replicated over time. Values of free and conjugated ubiquitin are means and standard errors of three replications. In the radioimmunoassay of free ubiquitin, data were on the linear portion of a standard curve (percentage of labeled ubiquitin bound to antibody *versus* log unlabeled ubiquitin in sample). Two controls, samples with antiserum omitted and samples with unlabeled ubiquitin omitted, were used.

Relative amounts of conjugated ubiquitin were based on values obtained from dilutions of control samples (22°C-treated root samples). The density of the slots of the various treatments then were compared with the density of the slots of the control treatments. A separate slot blot was used for each replication, and several subsamples of each dilution of a treatment were evaluated on each blot.

Reagents

Ubiquitin and protein A were from Sigma, carrier-free sodium iodide (^{125}I) and ENHANCE were from New England Nuclear, L-[4,5-^{3}H(N)]leucine was from American Radiolabeled Chemicals Inc., and bicinchoninic acid protein assay reagent and Iodogen were from Pierce. The histone monoclonal antibody was from Chemicon International, Inc. Ubiquitin was iodinated with chloramine-T (18), and protein A was iodinated with Iodogen (17).

RESULTS

Mean half-life of total protein in roots was 51 h at 22°C (R^2=0.73), 41 h at 32°C (R^2=0.63), and 23 h at 40°C (R^2=0.80). Thus, the rate of protein degradation more than doubled in roots from the low to high temperature.

Preliminary work using protocol B and a 2-min mixing time did not detect an increase in the levels of ubiquitin conjugates at high temperatures because conjugates were incompletely extracted. Increasing the mixing time to 5 min improved the conjugate extraction but still did not give maximum extraction, as illustrated in Figure 1.

Both protocol A and protocol B with a 5-min extraction detected increases in high molecular mass conjugates after the 42°C stress treatment compared with the 22°C treatment (Fig.

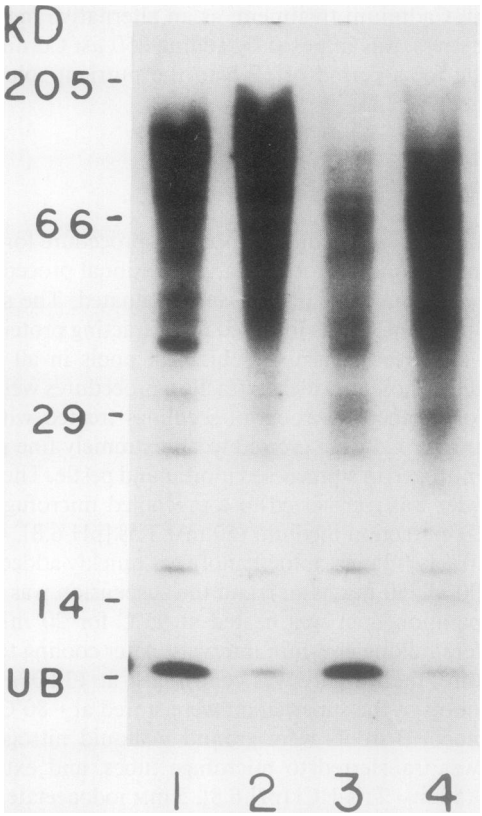

Figure 1. Radioimmunoblot comparing ubiquitin-conjugate extraction protocols. Roots were treated for 1 h at 22°C (lanes 1 and 3) or 42°C (lanes 2 and 4) followed by two different extraction protocols (A *versus* B). Equal protein (20 μg) was loaded on each lane, and the blot was probed with an ubiquitin antibody. In protocol A (lanes 1 and 2), root tissue was ground in liquid nitrogen and quickly mixed with hot extraction medium containing SDS and mercaptoethanol but no protease inhibitors, and the mixture was heated at 95°C for 20 min. After cooling, the mixture was centrifuged, and the resulting supernatant was used. In protocol B (lanes 3 and 4), ground root tissue was vigorously mixed for 5 min at room temperature with an extraction medium that contained SDS and several protease inhibitors but not mercaptoethanol. The mixture was then centrifuged, and the supernatant was mixed with a buffered solution containing SDS and mercaptoethanol, heated as above, and used in the experiment. Molecular mass markers are shown on the left. Additional details of the extraction procedures are in "Materials and Methods."

1). High molecular mass conjugates, however, were much more effectively solubilized by protocol A than protocol B. Ubiquitin conjugates with a molecular mass >100 kD are very prevalent in preparations from protocol A but not protocol B. Protocol B relied on protection by the thiol protease inhibitors, which precluded use of mercaptoethanol, whereas protocol A contained mercaptoethanol and used rapid heating of the extract to aid solubilization and minimize proteolysis of conjugates.

Roots treated at the control temperature (22°C) displayed a wide range of ubiquitin conjugates ranging from 23 to 180 kD (Fig. 2). Increasing the temperature of root treatment to 37°C distinctly increased the quantity of high molecular mass ubiquitin conjugates. The 42°C treatment also increased high molecular mass conjugates but, in contrast to 37°C, caused sharp reductions in low molecular mass ubiquitin conjugates (indicated by arrows at the right of the gel) and in free ubiquitin. The Cd treatment caused no large differences in ubiquitin pools.

Free ubiquitin declined markedly and conjugated ubiquitin increased significantly in roots as the treatment temperature was raised from 32 to 37 and 42°C (Table I). Temperatures from 22 to 23°C, however, had little consistent effect on either pool. Roots treated with Cd for 12 h at 22°C, in contrast, had elevated levels of both free and conjugated ubiquitin.

The 23-kD conjugate (lower arrow on right side in Fig. 2) is tentatively identified as an ubiquitinated histone. A calf thymus histone preparation containing about 1.5% uH2A and a root sample (22°C treatment) are compared in adjacent lanes on a radioimmunoblot in Figure 3A. The 23-kD conjugate in the root sample migrated with the uH2A in the histone sample.

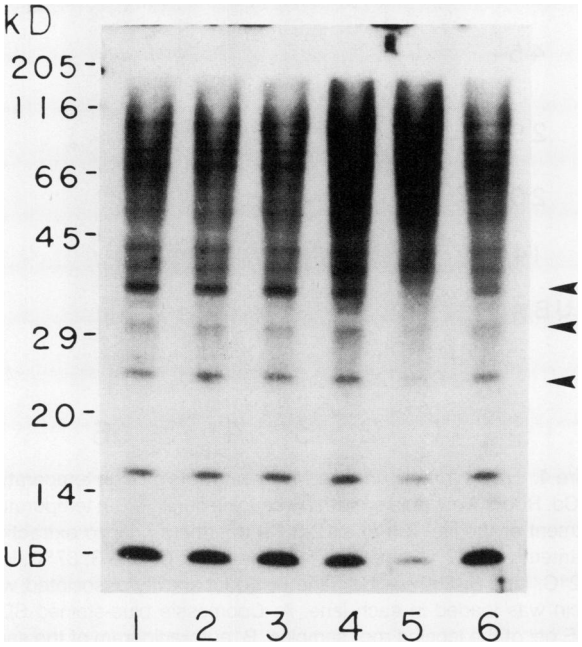

Figure 2. Radioimmunoblot of ubiquitin and its conjugates from wheat roots subjected to high temperature and Cd treatments. One-h treatments were imposed on roots prior to extraction. Treatments were: 22°C, lane 1; 27°C, lane 2; 32°C, lane 3; 37°C, lane 4; 42°C, lane 5, 12 h with 250 μM Cd, lane 6. Equal protein (20 μg) was applied to each lane, and blots were probed with ubiquitin antibodies. Arrows on the left indicate distinct ubiquitin conjugates. The free ubiquitin band is indicated by Ub. Molecular mass markers are shown on the left.

Table I. *Free and Conjugated Ubiquitin in Wheat Roots Treated at Five Temperatures or with Cd*

Free ubiquitin was measured by solution-phase radioimmunoassay, and conjugated ubiquitin was determined by solid-phase radioimmunoassay. Samples from roots treated at 22°C were considered the control. The relative amounts of conjugated ubiquitin in the other samples were determined by a standard curve made from the control sample.

Treatment	Free Ubiquitin	Conjugated Ubiquitin
	pmol Ub/mg protein ± SE	*% of control* ± SE
22°C	115 ± 5	100
27°C	126 ± 3	99 ± 2
32°C	113 ± 9	101 ± 8
37°C	96 ± 3	124 ± 4
42°C	82 ± 2	129 ± 11
Cd	137 ± 5	119 ± 12

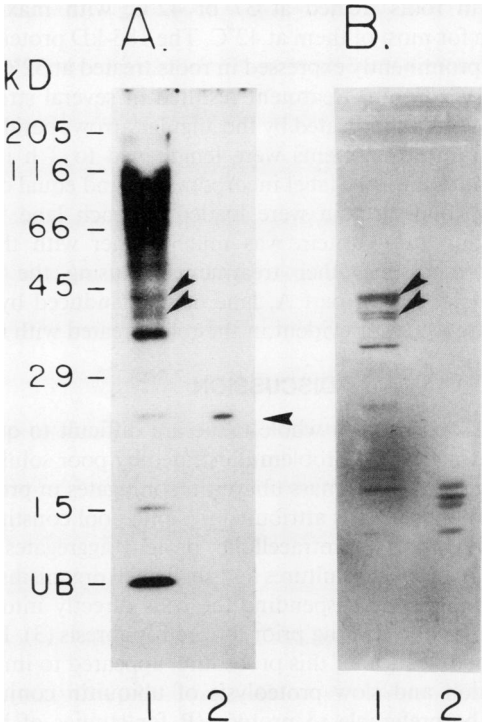

Figure 3. Blot of root sample and calf thymus histones probed with ubiquitin and histone antibodies. A, Radioimmunoblot probed with ubiquitin antibody. Samples are: lane 1, root sample (22°); lane 2, calf thymus histone sample. Horizontal arrow indicates uH2A from calf thymus and the corresponding band in the root sample. Angular arrows correspond with bands in part B. Molecular mass markers are shown at the left. B, Same blot as in part A that was reprobed with a monoclonal antibody to mammalian histones specific to H1, H2A, H2B, H3, and H4. Immunochemical staining was with alkaline phosphatase. Samples are identified as in part A. Angular arrows point to bands of similar migration in part A and part B.

To further verify that the 23-kD conjugate was a ubiquitinated histone, the blot was reprobed with a monoclonal antibody to mammalian histones as shown in Figure 3B. Neither the uH2A from calf thymus nor the 23-kD ubiquitin conjugate from the roots was recognized by this antibody. The core histones in wheat were easily recognized by the antibody, although some bands were faintly discernible. Several bands identified with the antibody matched bands of similar molecular mass on the blot probed with the ubiquitin antibody and have apparent molecular masses ranging from 42 to 38 kD. The major band with an apparent molecular mass of 33 kD may be histone 1 (H1) in the root sample.

Synthesis of HSP in roots exposed to high temperature is illustrated in Figure 4B. The HSP are indicated by arrows to the right of the autoradiogram. The apparent molecular masses of the proteins marked with horizontal arrows are (top to bottom) 185, 94, 76, 70, 59, 33, and 15 kD. These HSP occurred in roots treated at 37 or 42°C, with maximum expression for most of them at 42°C. The 185-kD protein that was most prominently expressed in roots treated at 32°C is an exception. Cadmium treatment resulted in several stress-induced proteins, as indicated by the angular arrows beside lane 6. Temperature treatments were lengthened to 3 h in this experiment to improve label incorporation, and equal counts instead of equal protein were loaded on each lane. Label incorporation into protein was much higher with the Cd treatment than the other treatments, causing the lower amount of protein in part A, lane 6. HSP induced by high temperature were not evident in the roots treated with Cd.

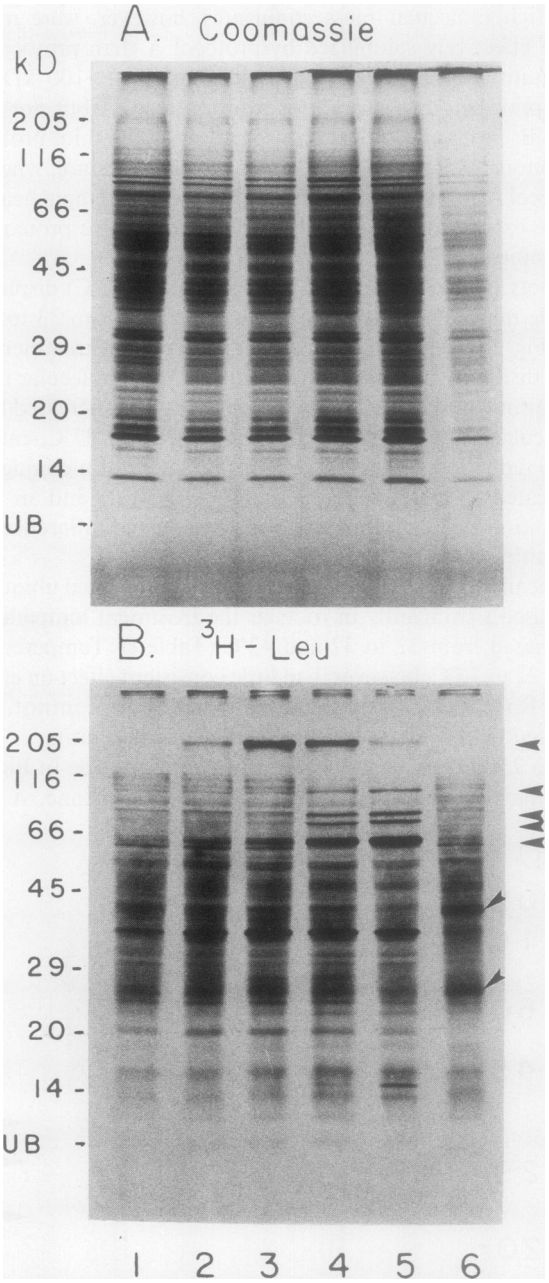

Figure 4. HSP synthesis induced in wheat roots by high temperature and Cd. Roots were labeled with ^{3}H-Leucine during a 3-h temperature treatment or the last 3 h of a 12-h Cd treatment prior to extraction. Treatments are 22°C, lane 1; 27°C, lane 2; 32°C, lane 3; 37°C, lane 4; 42°C, lane 5; 250 μM Cd, lane 6. Equal activity associated with protein was loaded in each lane. A, Coomassie blue-stained SDS-PAGE gel of the labeled root samples. B, autoradiogram of the same gel in part A. Horizontal arrows on the right mark specific heat-shock proteins. The angular arrows beside lane 6 mark stress-induced proteins that are specific to Cd treatment. Molecular mass markers are at the left.

DISCUSSION

Ubiquitin pools from whole tissue are difficult to quantitatively extract (16), a problem illustrated by poor solubilization of high molecular mass ubiquitin conjugates in protocol B. Poor solubilization is attributed to some pool constituents being components of intracellular protein aggregates (23). Ubiquitin pools in cell cultures and single-cell organisms have been determined by suspending the cells directly into SDS sample buffer and heating prior to electrophoresis (3). Protocol A, a modification of this procedure, appeared to improve solubilization and slow proteolysis of ubiquitin conjugates and may be preferable to protocol B for studies of higher plants.

Modulation of the ubiquitin pools in roots and simultaneous changes in cellular proteins support strongly that ubiquitin is involved in responses to elevated temperatures in higher plants as well as other organisms (4, 6, 12). Elevated temperatures injure plant proteins directly or indirectly by inactivating enzymes (28), stimulating accumulation of unprocessed peptides in the ER (8), changing peptide conformation (35), and disrupting membrane complexes (2). Injured intracellular proteins must either be degraded or repaired. Accelerated rates of protein breakdown and elevated levels of ubiquitin-protein conjugates (P_T-Ub) in stressed roots suggest a portion of these injured proteins (P_T) may be degraded by the ubiquitin proteolytic pathway as illustrated in Figure 5.

Concurrent induced synthesis of HSP and increased high molecular mass ubiquitin conjugates (P_T-Ub) by 37 and 42°C treatments is likely more than coincidental. Activation of HSP genes is due to accumulation of intracellular injured or aberrant proteins (1); a subset of these injured proteins is likely P_T. The HSP observed in roots treated at 37 and 42°C are very similar in apparent molecular mass to those described

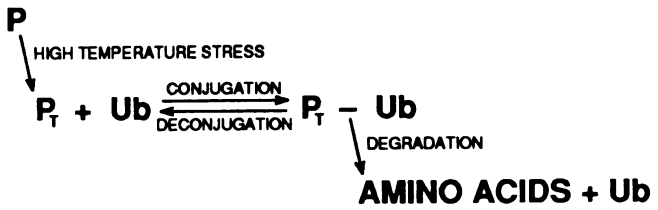

Figure 5. A model illustrating the dynamics of ubiquitin pools in wheat roots during high temperature stress. In this model, P represents protein; P_T, protein targeted for ubiquitination; Ub, free ubiquitin; P_T-Ub, ubiquitin conjugates. Pathways indicated by the arrows represent a process in which proteins are made susceptible to ubiquitination, ubiquitin conjugation, and degradation or deconjugation of ubiquitin conjugates. This model was adapted from that proposed originally by Haas (16).

by Necchi *et al.* (25), who also used wheat roots. The prominent 59 kD HSP (Fig. 3B) is prevalent in roots but not in coleoptiles (25).

Cellular levels of high molecular mass ubiquitin conjugates (P_T-Ub) reflect apparent rates of conjugation, deconjugation, and degradation. Conjugation is a result of several enzymatic activities, and the apparent rate of conjugation is determined by P_T, Ub, and ATP. If the conjugating enzymes are not rate-limiting, then an increase in the level of P_T would enhance synthesis of P_T-Ub and account for the increase in P_T-Ub in roots stressed at 37 and 42°C. This increase in P_T-Ub with high temperature stress is similar to responses in green algae (30) and avian (3) and mammalian cells (27). Enhanced amounts of P_T-Ub in stressed roots, if they represent an elevated steady-state level, would have a large accumulative effect on rates of protein degradation.

Alternative explanations for the increase in P_T-Ub in temperature-stressed roots cannot be excluded. The increase in P_T-Ub may represent an accumulation of conjugates, not an elevated steady state. If the activities of the enzymes responsible for degradation and/or deconjugation (Fig. 5) are inhibited at 37 or 42°C, then P_T-Ub may accumulate.

The 12-h Cd treatment, an alternative stress, induced increases in free and conjugated ubiquitin. Uncertainty about the degree of increase in P_T-Ub in response to Cd treatment remains, since the increase in P_T-Ub in Table I is not as evident in Figure 2. The elevated level of Ub, however, was not reflected in a reduced amount of P_T-Ub. Early Cd stress might have elicited a response similar to the temperature treatments, followed by increased ubiquitin synthesis to compensate for the ubiquitin being conjugated. Activation of cellular defenses against Cd diminished the protein being ubiquitinated and enhanced free ubiquitin, a phenomenon that is consistent with results on the ubiquitin pools following different periods of stress (3).

The amount of free ubiquitin in roots treated at 22°C was two to three times that in rat muscle cells on an equal protein basis (29). A large endogenous pool of free ubiquitin may explain the small increase in expression of a polyubiquitin gene in *Arabidopsis* after a 2-h heat shock (6). Roots treated at 42°C had 29% less free ubiquitin than roots treated at 22°C, which reflects the increase in P_T-Ub and suggests that a greater decline in free ubiquitin is required for enhanced expression of the polyubiquitin gene.

The ubiquitin conjugate with an apparent mass of 23 kD in Figures 2 and 3 is tentatively identified as a ubiquitinated histone based on the comigration of this conjugate and uH2A from calf thymus. The actual mass of mammalian uH2A is 22.5 kD (14). Our results do not distinguish whether this band is uH2A or uH2B. The sharp decline in the ubiquitinated histone and the other low molecular mass ubiquitin conjugates at 42°C is consistent with responses of animal cells exposed to heat shock (3, 27). Ubiquitinated histones in plants have not been described; however, their presence has been presumed.

The role of ubiquitinated histones in chromatin remains controversial (5). They are important in the structure of transcriptionally active chromatin (26), and their loss from roots treated at 42°C suggests that stress results in significant changes in chromatin structure.

ACKNOWLEDGMENTS

We thank Dr. Arthur L. Haas for the gift of the ubiquitin antibody and Dr. Gerald R. Reeck for the calf thymus and help with histone isolation.

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