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Phenotypical Temperature Adaptation of Protein Synthesis in Wheat Seedlings[1]

QUALITATIVE ASPECTS. INVOLVEMENT OF AMINOACID:tRNA-LIGASES

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ABSTRACT

Phenotypical temperature adaptation of protein synthesis in wheat (*Triticum aestivum* L.) seedlings is not affected by darkness (etiolation), by partial inhibition of protein biosynthesis (10^{-3} M fluorophenylalanine), or by changing the amino acid precursor and the radioisotope ([^{3}H]valine instead of [^{14}C]leucine). The temperature coefficient (μ), as well as the optimum temperature of *in vivo* protein synthesis, increases with rising preadaptation temperature, as normally observed. Protein turnover studies revealed that only proteins with a short half-life time ($t_{1/2}$ = 2 to 4 hours) are labeled to a measurable extent during the temperature adaptation experiments. A heat-labile protein has been detected and partially characterized by means of polyacrylamide gradient gels. Leucine:tRNA-ligase (EC 6.1. 1.4) from heat-pretreated wheat seedlings exhibits enhanced thermal stability. In Arrhenius curves, the upper transition point shifts from 30 to 34°C, depending on preadaptation temperature. Only the leucine:tRNA-ligase extracted from heat-adapted plants is stable when the enzyme extracts are subjected to a 34°C heat treatment.

The phenomenon of phenotypical, or capacity, temperature adaptation (17) of protein synthesis in wheat seedlings, 10 to 12 d old, has been characterized previously (22, 24). The optimum temperature, as well as the temperature coefficient (μ) of [^{14}C] leucine incorporation in the 'total protein' fraction, seems to be under the control of the preadaptation temperature. Both parameters show lowest values in case of cold-adapted plants, while heat-adapted plants exhibit the highest values.

The present investigation deals with the following aspects of the phenomenon. First, we considered it important to probe into the vulnerability, or stability, of this adaptation process against exogenous influences. Thus, dark-grown, etiolated wheat seedlings and fluorophenylalanine-treated plants, with a 50% inhibition of the rate of protein synthesis, were exposed to different temperature regimes. Second, protein degradation measurements were carried out. A correct assessment of temperature adaptation of protein synthesis requires a knowledge of the turnover rates of the protein moiety which is labeled under the specific conditions applied. Third, we intended to find out if temperature adaptation of protein synthesis is a universal phenomenon or if proteins with a deviating pattern of thermal stability can be identified. Fourth, a major goal of this study was to determine the molecular basis of temperature adaptation of protein synthesis. The amino acid tRNA-ligases (*i.e.* leucine:tRNA-ligase) were considered to be one possible target of primary adaptive temperature action for the following reasons: the tRNA aminoacylation reaction is known to be temperature-labile under many circumstances (11, 21); and it possibly controls the rate of protein biosynthesis (14, 28). Also, it is the only step in the protein biosynthesis sequence which can be studied without complicating interferences, because it takes place distant from the ribosome mRNA aggregates.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seed from spring wheat (*Triticum aestivum* L.) var. Kolobri (1978 harvest) was a gift from the F. von Lochow Breeding Company (Northeim, Federal Republic of Germany). Seedlings were cultivated for 10 d at 20 ± 0.5°C under continuous irradiation (approximately 100 $\mu E \cdot m^{-2} \cdot s^{-1}$; Sylvania cool-white fluorescent tubes). For all routine temperature treatments (4, 20, and 36°C, respectively) 10- to 12-d-old plants (shoots only) were used. Details have been published previously (22, 24).

Measurement of *in Vivo* Protein Synthesis Rates. Unless stated otherwise, labeled L-[U^{14}C]leucine was used (10^{-3} M, 0.33 $\mu Ci \cdot \mu mol^{-1}$). Incubation procedure, separation of the total protein fraction, and determination of the specific radioactivity (pmol leucine/mg protein) have been outlined earlier (22, 24).

Polyacrylamide Gradient Gel Electrophoresis. Five g wheat shoot material were frozen in a mortar under liquid N_2, powdered, and transferred into centrifuge tubes. All subsequent steps were carried out at 2 ± 1.0°C. After addition of 15 ml extraction buffer medium (25 mM Tris-Cl (pH 7.5), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol) and thawing, the homogenate was thoroughly extracted with an Ultra Turrax tissue homogenizer (Janke and Kunkel, Stauffen, Federal Republic of Germany). The slurry was squeezed through eight layers of gauze and centrifuged (40,000*g*, 15 min). The protein concentration of the crude extract was adjusted to 1 $mg \cdot ml^{-1}$ with buffer, using the Lowry test (12). Using ammonium-sulfate precipitation (cold-saturated $[NH_4]_2SO_4$ solution in 40% strength extraction buffer), the 40- to 80%-saturated ammonium sulfate fraction was obtained and pelleted (20,000*g*, 10 min). The protein was dissolved in 2 ml 40% strength extraction buffer and dialyzed for 20 h against 2 L of the same buffer. An occasional slight turbidity of the dialyzed samples was removed by centrifugation (40,000*g*, 15 min). Samples were stored at −20°C after addition of 0.6 ml glycerol (65%) per 1.8 ml protein solution.

Gel electrophoresis was performed on 4 to 30% polyacrylamide gel slabs (7.5 × 7.5 × 0.3 cm; Deutsche Pharmacia, Freiburg, Federal Republic of Germany), using Tris-Cl (pH 8.35) running buffer (90 mM Tris, 80 mM Na_2-tetraborate, 3 mM Na_2-EDTA). Each position was loaded with 66 μg protein in gels which had

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been preequilibrated (30-min prerun). Electrophoresis was performed at 7.5°C for 16 h under constant current (70 mamp/gel). The staining solution consisted of 0.9 g Serva-Blue R 250 (Serva, Heidelberg, Federal Republic of Germany) dissolved in 32 g TCA, 107 g sulfosalicylic acid, 268 ml methanol, and 604 ml distilled H_2O. After staining (30 min, 60°C), the slabs were destained in acetic acid:ethylacetate:ethanol:distilled H_2O (50:100:70:780). Gels were photographed, and banding patterns were scanned at 645 nm with a microdensitometer.

Estimation of Protein Degradation Rates. Substrate (vermiculite) sticking to the roots of the wheat seedlings was carefully, but cautiously, rinsed off before the plants were positioned in 100 ml beakers containing membrane-filtered tap water (pore size 0.2 μm, type 11307; Sartorius, Göttingen, Federal Republic of Germany) for 2 h. Then, the plants were transferred into the incubation solution (0.25 [0.50] mCi ^3H_2O/ml in sterile-filtered tap water). The beakers were covered with Parafilm around the emerging shoots to minimize direct evaporation. The beakers were placed in a transparent chamber inside a growth cabinet (20°C; continuous irradiation, 70 $\mu E \cdot m^{-2} \cdot s^{-1}$). The incubation chamber was connected to an air pump generating a constant rate of air exchange (2 $L \cdot min^{-1}$) to improve reproducibility of ^3H_2O incorporation rates. Following ^3H_2O incubation, the seedlings were transferred back into ^1H_2O until they were harvested in batches of four, according to the time schedule of the experiment. The plant material was rinsed; roots were removed; and shoots were weighed, frozen in liquid N_2, and pulverized. All further procedures were done as described by Humphrey and Davies (8), measuring the ^3H activity in C-2 of amino acids from protein hydrolysates after racemization with acetic anhydride.

Leucine:tRNA-Ligase. All operations were carried out at 2.0 ± 1°C. In a precooled mortar, 5 g wheat shoots were homogenized with 15 ml of extraction buffer medium (after Kanabus and Cherry [10]), consisting of 40 mM 0.1 M Tris-HCl (pH 7.9), 40 mM $MgCl_2$, 10 mM β-mercaptoethanol, 1 mM GSH, 10 μM phenylmethylsulfonylfluoride, and 1 μM L-leucine. Each sample also contained 4 g quartz sand and 1.5 ml glycerol (65%). The homogenate was squeezed through eight layers of gauze and centrifuged (40,000*g*, 15 min), and the supernatant was filtered through Miracloth to remove most of the lipid material. Cold-saturated ammonium sulfate solution in K-phosphate (pH 7.9) (25 mM $K_2HPO_4 \cdot 3\ H_2O$/KH_2PO_4, 10 mM β-mercaptoethanol, 10 mM phenylmethylsulfonylfluoride, 1 μM L-leucine) was slowly added to establish 65% saturation. After 60 min, the precipitated protein was pelleted (20,000*g*, 10 min) and dissolved in 20 ml K-phosphate (see above). The enzyme solution was used immediately for the leucine:tRNA-ligase assays. The reaction mixture consisted of 15 μmol (50 μl) Tris-Cl (pH 8.1), 80 nmol (10 μl), $MgCl_2$, 0.5 μmol (10 μl) ATP-Na_2, 0.5 μmol (10 μl) GSH, 15 nmol 1 μCi (10 μl) L-[U^{14}C]leucine, 166 μg (100 μl) wheat germ tRNA mixture (Sigma). All components were added to microreaction tubes in the above order. Tests were run in triplicate with two blanks, buffer replacing ATP. When the test temperature (25°C, unless stated otherwise) was reached (2 min), the reaction was started by adding 10 μl enzyme solution and terminated after 3 min with 1 ml ice-cold 10% TCA containing 10 mM L-leucine. The precipitate was collected on membrane filters (pore size, 0.4 μm; type 11106; Sartorius) and washed with ample volumes of 10% TCA:10 mM L-leucine, 5% TCA, ethanol:diethylether (3:1 v/v), and dry diethylether. The air-dried filters were counted after addition of 3 ml scintillation cocktail composed of 100 mg dimethylpopop, 4 g paraterphenyl, and 1 L toluene. Temperature curves are based on at least six independent series of experiments. Regression statistics were computed using the computer facilities of the university (Regionales Rechenzentrum an der Universität zu Köen, Köln).

RESULTS

The first series of experiments was designed to evaluate the dependence of the phenomenon of phenotypical temperature adaptation of protein synthesis on the incubation conditions. The rate of incorporation of exogenously supplied L-[^{14}C]leucine into the total protein fraction is independent of concentration within the range $5 \cdot 10^{-5}$ M to 10^{-3} M (Fig. 1). This indicates that, under 'swamping' conditions recommended for such incubation experiments (7), involving unphysiologically high concentrations of precursor, no deleterious effect on protein synthesis rates can be observed as far as wheat seedlings and leucine are concerned. The use of L-[^{3}H]valine instead of L-[^{14}C]leucine makes no difference with respect to the pattern of temperature adaptability of *in vivo* protein synthesis (Fig. 2; Table I). This result, complemented by analogous data with L-[^{14}C]phenylalanine (not shown), strengthens the conclusion (24) that the protein synthesis rates routinely measured with leucine are, indeed, valid and representative.

The following experiments show that the adaptive influence which temperature exerts on protein biosynthesis rates is consistent. Fluorophenylalanine inhibits protein synthesis *in vivo* about 50% when wheat seedlings are incubated simultaneously with equimolar concentrations (10^{-3} M) of this phenylalanine analog and L-leucine (Fig. 1). The pattern of temperature adaptation of protein synthesis is not affected under these adverse conditions. A comparison with the standard temperature adaptation curves clearly shows that the typical increase with rising preadaptation temperature of the temperature coefficient (μ) and the optimum temperature (expressed by the rate quotient 35°C/20°C) remains unaffected (Fig. 2; Table I). The same holds true for dark-grown, etiolated wheat seedlings as compared to light-grown plants (Fig. 2; Table I).

Several conditions require that the incubation time (15 min) be mimimized when *in vivo* protein synthesis rates are being measured via incorporation of L-[^{14}C]leucine (10^{-3} M): the unphysiologically high precursor concentration; the submersed condition of the wheat shoots during incubation; precursor recycling; and the possibility of temperature readaptation of the plants taking place during the incubation period. Experiments looking at turnover rates of wheat protein show the existence of a protein moiety with a half-life time of a few h, and it is safe to assume that, during the 15-min incubation period of the *in vivo* protein synthesis experiments, only rapidly turning over proteins have a chance to become labeled to a measureable extent. Even ^3H_2O incubation periods of

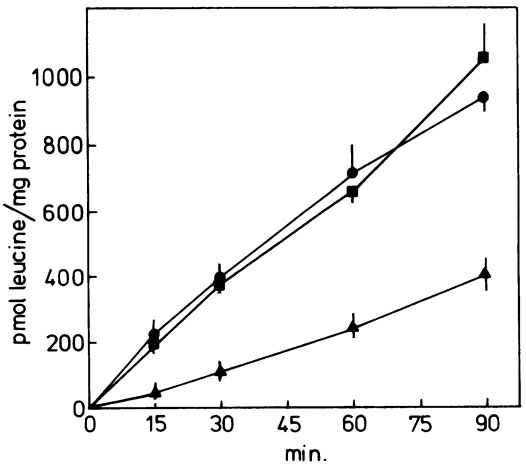

FIG. 1. Time curves for L-[^{14}C]leucine incorporation into wheat protein. (●), 10^{-3} M L-leucine; (■), 5×10^{-5} M L-leucine; (▲), 10^{-3} M L-leucine + 10^{-3} M fluorophenylalanine. Wheat seedings were 12 d old and preconditioned to 20°C (bars, SE).

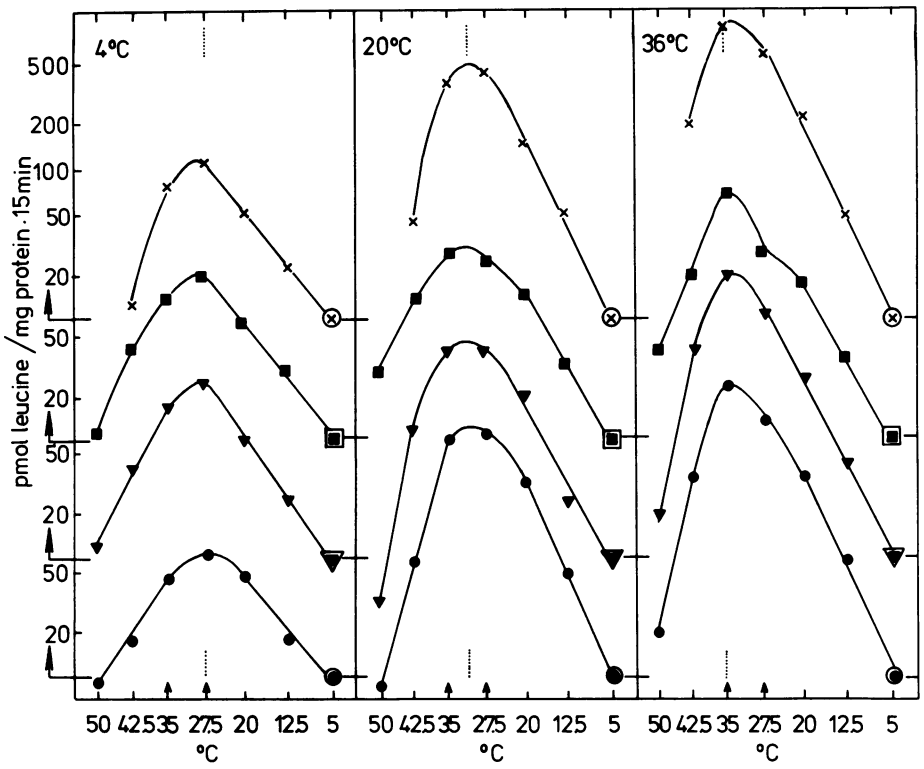

FIG. 2. Arrhenius plots of temperature characteristics for amino acid incorporation into protein of wheat seedlings, preadapted for 2 d at 4, 20, and 36°C, respectively. Curves were standardized with respect to the 5°C-values (encircled). L-[^{14}C]Leucine (10^{-3} M), light (×); L-[^{14}C]leucine (10^{-3} M) + *p*-fluoro-L-phenylalanine (10^{-3} M), light (■); L-[^{3}H]valine (10^{-3} M), light (▼); L-[^{14}C]leucine (10^{-3} M), dark (●).

Table I. *Temperature Characteristics of Protein Synthesis in Wheat Seedlings Preadapted to Low (4°C), Medium (20°C), and High (36°C) Temperature for 48 Hours*

Values were extracted from Figure 2, and statistical data were calculated by regression analysis.

	Temperature Preadaptation Treatment					
	20 d at 20°C + 48 h at 4°C		20 d at 20°C + 48 h at 20°C		20 d at 20°C + 48 h at 36°C	
Incubation Conditions	Temperature coefficient (correlation coefficient)	Protein synthesis, at 35/20°C	Temperature coefficient (correlation coefficient)	Protein synthesis at 35/20°C	Temperature coefficient (correlation coefficient)	Protein synthesis at 35/20°C
	μ	*ratio*	*μ*	*ratio*	*μ*	*ratio*
[^{14}C]Leucine, 10^{-3} M, light	13.0 ± 1.0 (0.965)	1.36	21.3 ± 0.7 (0.992)	1.77	25.3 ± 2.4 (0.945)	2.66
[^{3}H]Valine, 10^{-3} M, light	13.6 ± 1.1 (0.948)	1.33	17.3 ± 0.9 (0.983)	1.56	17.9 ± 1.0 (0.980)	2.65
[^{14}C]Leucine, 10^{-3} M; p-fluoro-DL-phenylalanine, 10^{-3} M; light	14.4 ± 1.1 (0.959)	1.47	19.1 ± 1.7 (0.961)	1.60	19.5 ± 0.5 (0.993)	3.13
[^{14}C]Leucine, 10^{-3} M, dark	12.1 ± 1.1 (0.924	0.96	22.6 ± 0.8 (0.989)	1.58	23.6 ± 1.2 (0.973)	2.63

6 and 18 h, respectively. predominantly label proteins with half-life times ($t_{1/2}$) of 1.6 to 3.7 h (Fig. 3A). A 15-min $^{3}H_2O$ incubation period, which would be desirable in this context, is beyond our experimental possibilities, because of the extremely high amounts of labeled material needed. Only an incubation time of 48 h yields an average half-life time of about 73 h, a value which corresponds well with the turnover rates for total wheat protein (Fig. 3B) reported elsewhere (5).

The question arises as to whether all proteins participate in a like manner in high temperature adaptation of protein synthesis or whether it is possible to separate distinct proteins by gel electrophoretic techniques (*i.e.* on 4 to 30% polyacrylamide gradients gels) which exhibit distinct patterns in response to temperature. PAGE[3] of nondenatured soluble wheat protein (40 to 80% ammonium sulfate fraction) yields one single discernible protein band which is considerably reduced, although not completely erased, under the influence of a 48-h heat treatment (36°C) of the seedlings (Fig. 4). The protein in question, assuming that it is, indeed, a single protein from the narrowness and sharpness of the respective zone on the gel, has a mol wt of 254,000 daltons (Fig. 4). The strong decrease in the amount of this protein at 36°C is complete within 4 h (Fig. 5). Restoration of the original level takes place within about the same time when 36°C plants are shifted back to 20°C (Fig. 6). Cold treatment has no effect (Figs. 5 and

[3] Abbreviation: PAGE, polyacrylamide gel electrophoresis.

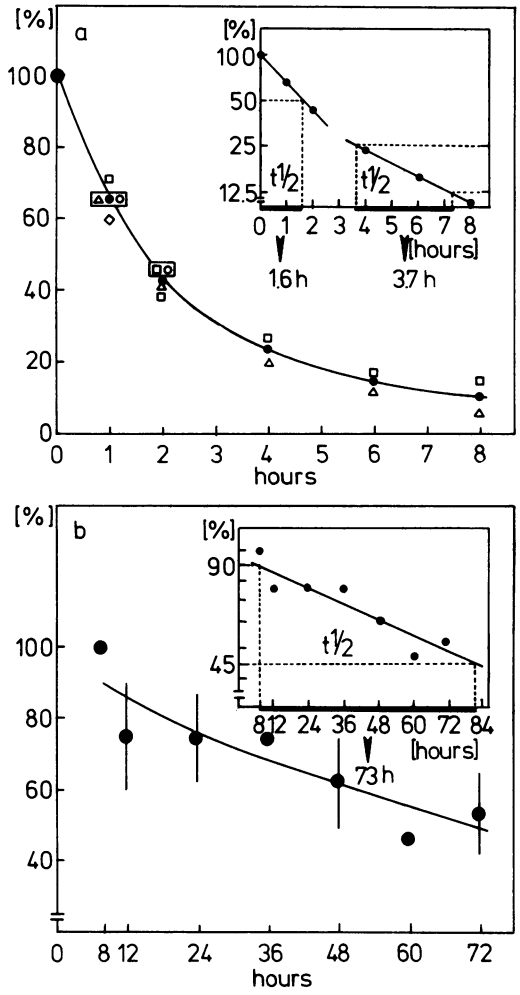

FIG. 3. Protein degradation curves. a, Protein was labeled with ^3H_2O (0.5 mCi ml^{-1}) for 6 h (◇, ○) or 18 h (□, △). The specific radioactivity of ^3H at C2-position of amino acids from hydrolyzed soluble protein at zero time after transfer of the wheat seedlings back from ^3H_2O into H_2O was set 100%. Inset, Logarithmic plot of same data (average values, ●) for calculation of the half-life time ($t_{1/2}$). b, Protein was labeled with ^3H_2O (0.25 mCi ml^{-1}) for 48 h (●); bars, SE. For '36 h' and '60 h' data, a SE was not calculated because only two independent values were available, otherwise: $n = 9$. Inset, Logarithmic plot of the same data. The regression line was computed for calculating $t_{1/2}$.

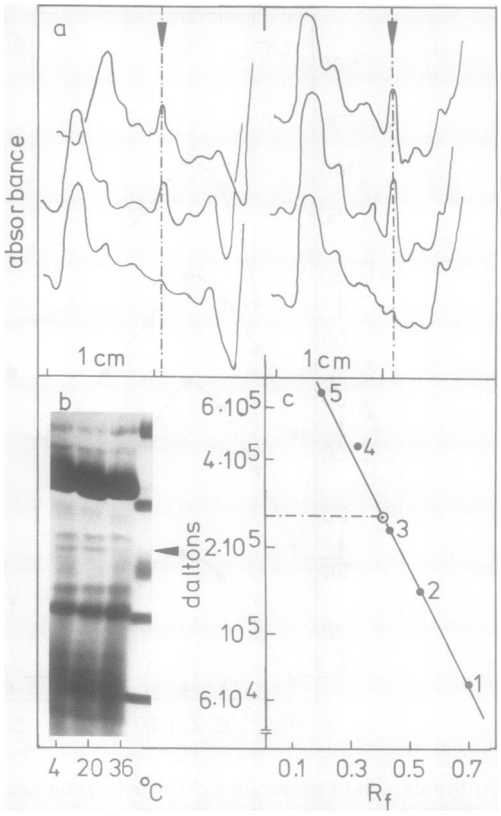

FIG. 4. a, Microdensitometer tracings from two independent experiments (a, b) of 4 to 30% polyacrylamide-gradient gels loaded with soluble protein (30–80% ammonium sulfate fraction) from cold-pretreated plants (48 h, 4°C; upper curves); heat-pretreated plants (48 h, 36°C; lower curves); and plants adapted to 20°C; middle curves). Arrow, heat-labile band. Absorbance was recorded at 645 nm. b, Photograph of polyacrylamide-gradient gels loaded with soluble protein (30–80% ammonium sulfate fraction) from 12-d-old wheat seedlings pretreated for 48 h at 4, 20, and 36°C. Arrow, heat-labile protein band. c, Estimation of the mol wt for the heat-labile protein band. Standards were: 1, albumin (67,000 mol wt); 2, ceruloplasmin (132,000 mol wt); 3, catalase (240,000 mol wt); 4, apoferritin (443,000 mol wt); 5, L_2-macroglobulin (820,000 mol wt).

6). The degradation kinetics of this protein are consistent with the degradation curve of short-lived proteins (Fig. 3A).

Prior to studying the temperature characteristics of leucine:tRNA-ligase, all assay conditions had to be optimized with respect to substrate and cofactor saturation, thus ensuring linearity and proportionality of the reaction over the whole temperature range up to the presumed upper transition point of thermal stability of the ligase. Based on substrate saturation curves and a pH-curve (not shown), test conditions have been worked out which meet these preconditions. It can be recognized that, at 30°C, the reaction rate is linear for 3 min and strictly proportional to the amount of enzyme (Fig. 7).

The temperature curves for the aminoacylation reaction of leucine:tRNA ligase for 4, 20, and 36°C plants are depicted in Figure 8. The corresponding Arrhenius plots are given in Figure 9. The enzyme obtained from heat-adapted wheat seedlings exhibits significantly increased thermal stability, although the optimum temperature (35°C) is the same in all cases. However, the activity ratio 35°C/20°C (Fig. 8, inset) is markedly higher in the case of 36°C plants than it is for the enzyme obtained from seedlings not heat-pretreated. Further, the temperature limit for heat inactivation of the enzyme is approximately 34°C in the case of 36°C plants but only 30°C in the case of 4 and 20°C plants (Fig. 9). The determination of the (upper) transition point of Arrhenius curves is considered to be a more accurate measure for the thermal stability of enzymes than is optimum temperature (1).

To confirm this admittedly small difference, enzyme extracted from preadapted low, medium, and high temperature plants was subjected to an *in vitro* heat treatment at the supposedly critical temperature of 34°C for various periods of time, followed by an enzyme assay at 15°C (Fig. 10). No loss in enzyme activity occurred in the case of leucine:tRNA-ligase from 36°C plants. However, one-third of the original activity was lost within less than 5 min, when the enzyme from 4 and 20°C plants was heat-treated. In any case, two-thirds of the original enzyme activity were retained. These inactivation curves certainly do not resemble the normal time course of thermal inactivation/degradation of amino acid:tRNA-ligases (11, 20).

In contrast to these temperature-induced differences in the optimum region, the temperature coefficient (μ) (=Arrhenius activation energy) for the aminoacylation reaction of leucine:tRNA-ligase is constant (38.5 kJ·mol^{-1}) and independent of the preadaptation temperature of the wheat seedlings (Fig. 9). For comparison, the μ values for *in vivo* protein synthesis vary between 54

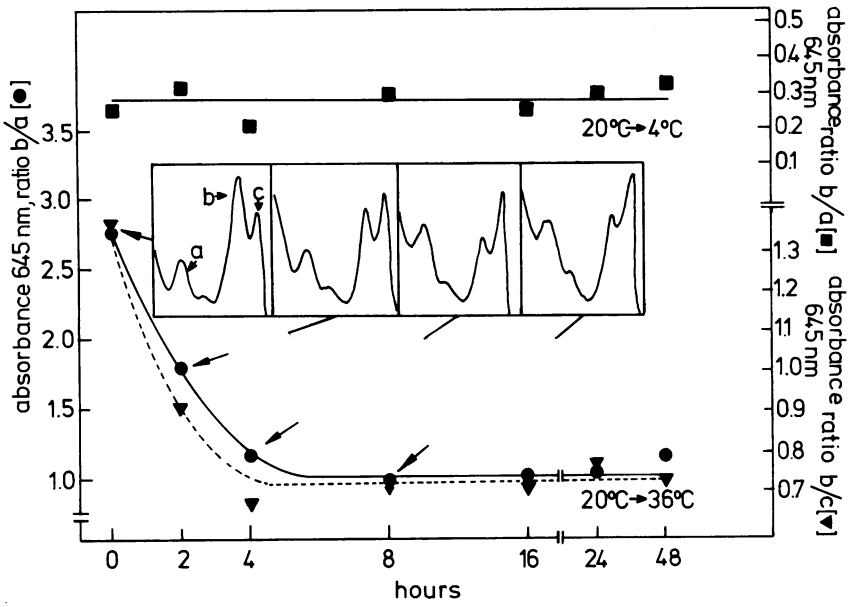

FIG. 5. Time-curve ('kinetics') 20°C, 10 d → 36°C. Time in h for the decrease in peak intensity of the heat-labile protein band (inset, b) in comparison to reference bands (inset, a, c). The ratios b/a (●) and b/c (▼) yield practically in the same curve. Also included is an experiment 20°C, 10 d → 4°C, time in h (■).

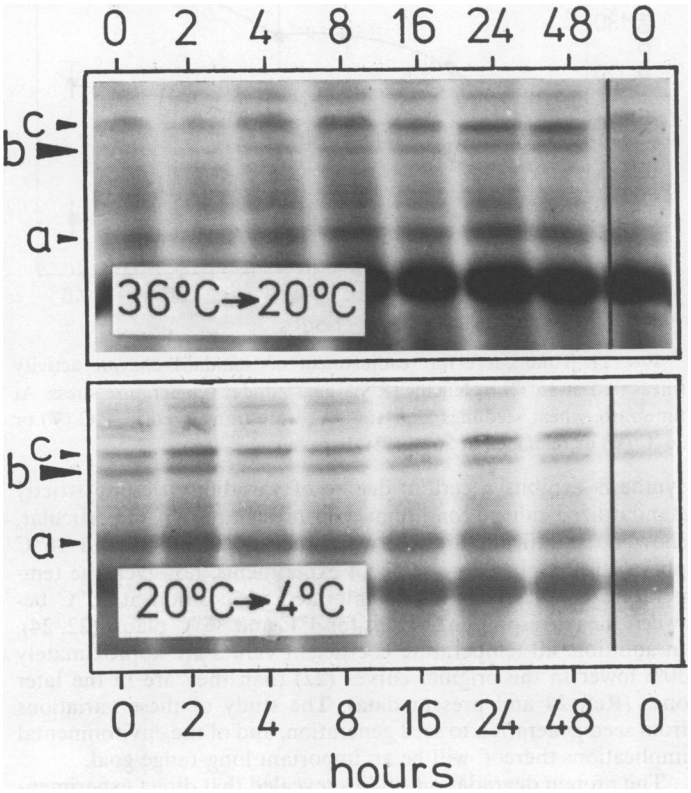

FIG. 6. Photographs of polyacrylamide-gradient gels showing a time series for 'readjustment' of protein band b from the 36°C-status to the 20°C-status (see Figs. 4 and 5 for details). A time series 20°C → 4°C is included for comparison.

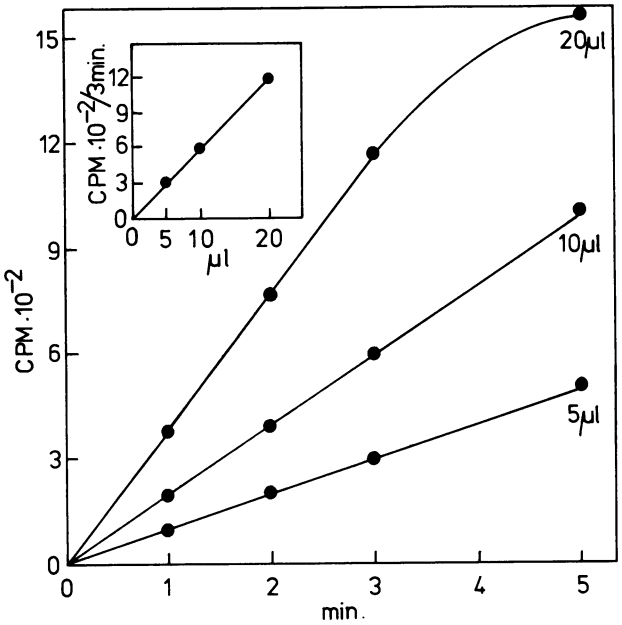

FIG. 7. Demonstration of 'V_{max}-conditions' at 30°C: linearity with time and proportionality between amount of enzyme and reaction-rate (inset) for the aminoacylation reaction of leucine:tRNA-ligase.

and 96 kJ·mol^{-1}, depending on preadaptation temperature (Table I).

Comparing the enzyme activities from 4, 20, and 36°C plants, at whatever assay temperature, it is evident (Fig. 8) that the leucine:tRNA-ligase activity is lowest at a preadaptation temperature of 20°C and highest in 36°C plants. The 4°C plants assume an intermediate position. Time curves for this vertical displacement of the temperature curves show (Fig. 11) that the readjustment of the enzyme level follows rather rapidly upon the shift in preadaptation temperature. Within only 3 h, 50% of the final activity level has been reached. This value includes a 1-h lag-phase.

DISCUSSION

Phenotypical temperature adaptation of *in vivo* protein synthesis in wheat seedlings is not adversely affected by etiolation. This physiological status, which is characterized by a largely different overall pattern of cell metabolism, includes a pronounced reduction of the rate of protein synthesis and substantial shifts in (enzyme) protein patterns (8, 19). Further, partial inhibition of protein synthesis by fluorophenylalanine does not influence the

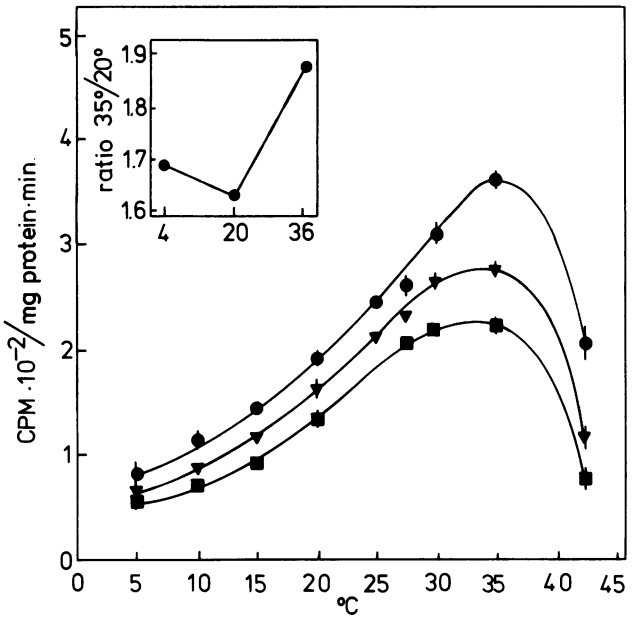

FIG. 8. Temperature curves for the aminoacylation reaction of a crude enzyme preparation of leucine:tRNA-ligase from 10-d-old wheat seedlings, temperature-pretreated for 48 h at 4°C (▼), 20°C (■), and 36°C (●), respectively. Inset, ratio of reaction rates 35/20°C as a function of preadaptation temperature of wheat seedlings. Data were standardized on the average of the 15°C-values (bars, SE).

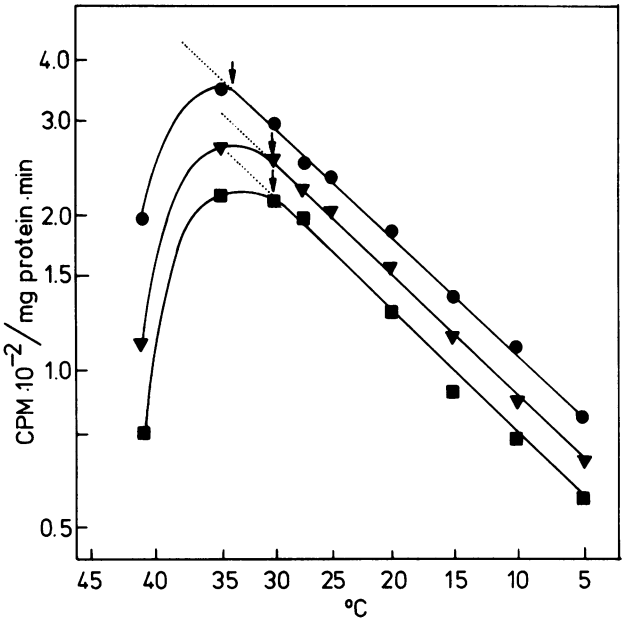

FIG. 9. Arrhenius plots of the data from Figure 8. Leucine:tRNA ligase from 4°C (▼), 20°C (■), and 36°C (●) plants exhibits a uniform Arrhenius activation energy: E_a = 38 kJ mol^{-1} (9.2 kcal mol^{-1}). The dotted lines mark the upper transition point of temperature stability.

adaptation pattern. In our opinion, these results suggest that the primary target(s) for temperature adaptation is located within the protein synthesis apparatus, itself, rather than being only loosely connected. In the latter case, one would expect to see consequences of some kind on the temperature adaptation phenomenon by such rigorous treatments.

One element of instability within the adaptation process becomes apparent, however, when the Arrhenius curves presented here are compared with those published earlier (Fig. 5 in Ref. 22; Fig. 3 in Ref. 24). The temperature coefficient (μ) of *in vivo* protein

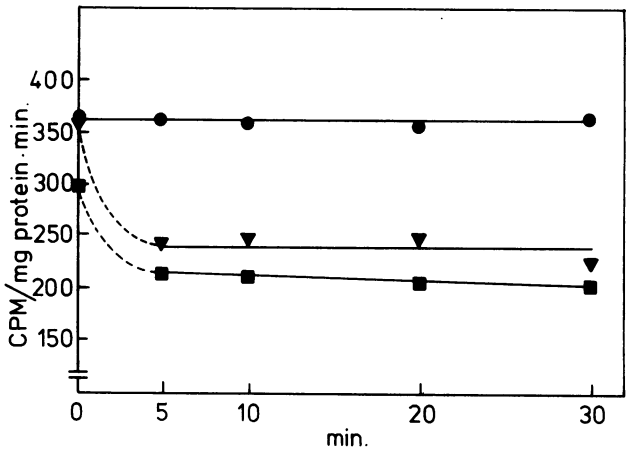

FIG. 10. Temperature inactivation curves for leucine:tRNA-ligase. Enzyme extract from wheat seedlings preadapted to 4°C (▼), 20°C (■), and 36°C (●), respectively, was heat-treated at 34°C for time intervals 0 to 30 min, before enzyme activity was assayed at 15°C.

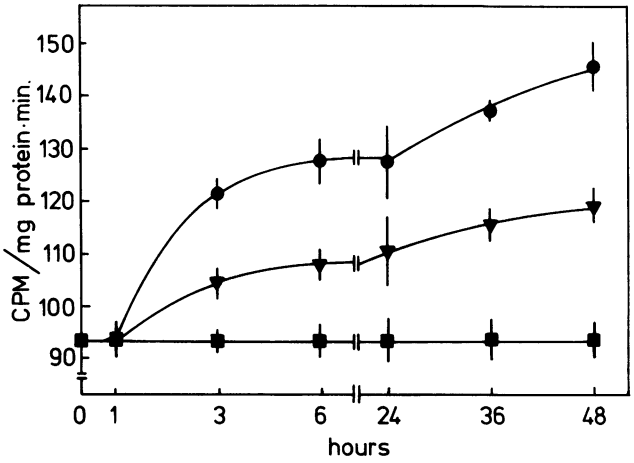

FIG. 11. Time curve for readjustment of 'standard'-enzyme activity (measured at 15°C) of leucine:tRNA-ligase under temperature stress. At time zero, wheat seedlings kept at 20°C, were transferred to 4°C (▼) or 36°C (●) or remained at 20°C (■) (bars, SE).

synthesis exhibits a certain degree of variability despite strictly standardized culture conditions. Our present results, in particular, show no significant difference between 20°C plants and 36°C plants. In the preceding series of experiments, however, the temperature coefficient holds an intermediate position at 20°C between the corresponding values for 4°C and 36°C plants (22, 24). In addition, all temperature coefficient values are approximately 30% lower in the original curves (22) than they are in the later ones (Ref. 24 and present data). The study of these variations from seed generation to seed generation, and of the environmental implications thereof, will be an important long-range goal.

The protein degradation studies revealed that direct experimental evidence for the existence of phenotypical temperature adaptation of protein synthesis in wheat is confined to short-lived proteins with an average half-life time of a few h and, thus, perhaps to a minority of cell proteins (7, 8). In light of this result, one earlier statement has to be reconsidered: 'A readaptation process which takes place within 1 or a few h after a temperature shift has been initiated can hardly be based on *de novo* synthesis of certain enzymes and/or protein factors of the protein synthesis apparatus...' (24). Apparently, temperature-induced isozymic replacement can no longer be excluded. Instead, it has to be envisaged as one possible adaptation mechanism, even in the case of quite rapid readaptation processes, such as readjustment of opti-

mum temperature of protein synthesis (24).

It has long been recognized that gel electrophoretic profiles of plant protein change gradually according to long-term seasonal temperature fluctuations (2, 3, 18). Few data are available, however, dealing with rapid changes in protein banding patterns as a result of temperature shifts (25, 27). The short-lived, heat-labile protein which has been detected by gradient PAGE may possibly play an important role in the investigation of the molecular basis of high temperature influence on wheat—once it has been identified. On the other hand, the same data also suggest that no across the board changes in protein composition of the seedlings occur when temperature is increased up to the heat limit of these plants. Wheat seedlings do not survive at 37 to 38°C. The results presented here are complemented by isoelectric focusing data (pH gradient 3–10), in which none of 22 separated bands is temperature-sensitive (data not shown). Admittedly, the resolution power of any one-dimensional gel electrophoretic technique does not match the complex composition of any total protein extract from plants. Nevertheless, it is safe to conclude that a large majority of proteins participate in the adaptive enhancement of the rate of protein synthesis at high temperature.

No strong experimental evidence can be presented yet which would allow one to propose distinct biochemical mechanisms underlying the temperature effects reported here for the aminoacylation reaction of leucine:tRNA-ligase. One has to be aware of the fact that the procedures of enzyme extraction and the replacement of the natural cytochemical environment by artificial test conditions might lead to *in vitro* temperature effects which are only a more or less modified reflection of the *in vivo* temperature effects they are based upon. In this respect, the results obtained by the 34°C, *in vitro* heat treatment of the leucine:tRNA-ligase are, nevertheless, of some value.

Unexpectedly, no constantly progressing thermal degradation/inactivation curve was obtained for enzyme from plants which were not heat-adapted. A very rapid loss (≦5 min) of about one-third of the initial activity is followed by the attainment of a lower stable level of enzyme activity. It suggests that this kind of inactivation kinetics could be the manifestation of a conformational change within the leucine:tRNA-ligase molecule, which does not occur when the wheat seedlings have been previously heat-adapted. The 36°C pretreatment of the plants might induce the formation of a more heat stable (iso-) enzyme population (4, 13, 23). Or, perhaps, under involvement of ATP (11, 15) or substrate (14), the conformation of the preexisting amino acid:tRNA-ligases is stabilized. Regardless of which mechanism turns out to be operative, it might concern only one or two of the three cell compartment-specific leucine:tRNA-ligases (10, 26). The other(s) may already be heat-stable at 34°C.

Both cold- and heat-pretreatment of the wheat seedlings cause a rise in enzyme activity·mg^{-1} protein, as compared to 20°C plants. The total protein concentration remains practically constant under all temperature regimes (22, 24). Hence, the increase in specific activity cannot be due to a decreased level of other (nonligase) proteins upon temperature treatment. Tentatively, we interpret these shifts, which take place within a few h, as temperature-controlled changes in the amount of enzyme. However, only more rigorous (*e.g.* immunological) studies and the knowledge of the turnover time of leucine:tRNA-ligase can substantiate this interpretation.

The facts that no differences in activation energy could be found between leucine:tRNA-ligase from 4, 20, and 36°C plants and that activation energy is much lower than the corresponding temperature coefficient values for *in vivo* protein synthesis exclude the possibility that the adaptive differences of the temperature coefficient of protein biosynthesis are derived from temperature-induced alterations of activation energy of the aminoacylation reaction.

Is the upper temperature limit of *in vivo* protein synthesis in wheat seedlings controlled by the pronounced thermal lability of the leucine (aminoacid):tRNA-ligases? Is the ability of the temperature optimum of protein biosynthesis to undergo temperature adaptation correlated with the shifts occurring in the optimum temperature region of the ligases? On one hand, *in vivo* protein synthesis and leucine:tRNA-ligase are both characterized by a remarkably low optimum temperature region at approximately 30 to 35°C and both exhibit temperature adaptability in the optimum region. On the other hand, the optimum temperature of protein biosynthesis varies from 27.5 to 35°C instead of 30 to 34°C, as in the case of leucine:tRNA-ligase. And, it is the upper transition point of thermal stability—and not the parameter optimum temperature, itself—of leucine:tRNA-ligase which readjusts. We think our data favors the assumption that a correlation between the two adaptation phenomena indeed exists. Certainly, it is a complex series of interactions which connects them, as indicated by the inconsistencies in detail.

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