Preadaptation of Protein Synthesis in Wheat Seedlings to High Temperature

MANFRED WEIDNER AND CORINNA ZIEMENS
Botanisches Institut der Universität Köln, Gymhofstrasse 15, Germany

Received for publication January 9, 1975 and in revised form June 9, 1975

ABSTRACT

The optimum temperature of protein synthesis in wheat seedlings (Triticum aestivum L.), measured as \(^{14}C\)-leucine incorporation, depends on the growing temperature. Plants grown at reduced temperature (4 C) reach their optimum at 27.3 C, whereas plants kept at 36 C have the highest rate of protein synthesis at 33 C. The transition is gradual. The activation energy of protein synthesis for seedlings grown at medium or reduced temperature is lower (about 11 kcal/mole), than for plants grown at higher temperatures (15 kcal/mole). The decline of the rate of protein synthesis beyond the temperature optimum is also affected by the growth temperature: only plants kept at 30 or 36 C show a sharp decrease with increasing slope; plants kept at 4, 10, and 20 C exhibit a linear and comparatively moderate decline.

Study of the temperature adaptations of organisms, including plants, has two aspects: the study of climatic ecotypes which are investigated to find out how genetic adaptation to varying temperature conditions operates (2, 3, 23, 29); and the study of a so-called capacity adaptation, which occurs when the temperature-dependence curve of any physiological or biochemical parameter is influenced by a temperature pretreatment (acclimation) of the experimental organism (26). Obviously the ability for capacity adaptation is genetic. Much of the interest in capacity adaptations of plants is focused on extreme temperatures: cold and heat hardening. Theory for frost resistance and hardiness is relatively far advanced (9), but the capacity adaptation to high temperatures still lacks any sound molecular explanation, although thermal denaturation of proteins and nucleoproteins (25) is generally assumed to determine the upper temperature limits for life.

The basic problem of reaction-rate compensation to temperature changes is one of regulating the rates of enzyme activity. Three major ways seem important: (a) changes in the concentration of preexisting enzymes; (b) changes in the types of enzymes present in the system, i.e. temperature-specific isoenzyme patterns; and (c) modulation of the activities of preexisting enzymes, e.g. by alteration of activation energies and substrate-binding affinities (Km) (10). Enzyme or protein concentrations are influenced by several interacting and often opposing factors. As temperature increases, denaturation increases, but so does rate of protein synthesis, as well as proteolytic and other inactivating metabolic activities. Below a critical temperature range, metabolic balance is preserved, but above some temperature threshold, thermal denaturation and metabolic degradation of protein become increasingly important. When protein synthesis and/or other adaptive mechanisms can no longer compensate for this metabolic stress, breakdown of metabolism will occur. Plants able to maintain high rates of protein synthesis at higher temperatures may be better able to cope with heat stress. The essential adaptation must be a special type: namely, "inverse compensation," in which case adaptation consists of a stimulation (instead of inhibition) of any experimental parameter by temperature increase (26, 30).

To investigate this possible means of temperature adaption, wheat seedlings were used to study the effects of different temperature pretreatments (i.e. acclimation temperatures) on the temperature characteristics of protein synthesis measured as \(^{14}C\)-leucine incorporation. Optimum temperatures for protein synthesis and temperature coefficients were estimated from Arrhenius plots. The term "activation energy" is used in a broad sense, although strictly speaking it should be reserved for single reactions, and should correctly be termed activation enthalpy, because the entropy of activation cannot be determined with the methods applied in this investigation.

MATERIAL AND METHODS

Plant Material and Growth Conditions. Spring wheat (Triticum aestivum var. Kolibri, 1972 harvest) was obtained from a commercial grower (H. Rausch, Meckenheim). Seeds were planted in flat culture trays on about 1 cm of moist sand and covered with a glass plate for the first 5 days. To ensure a constant nitrogen supply over an extended period of time (probably important in a study of protein synthesis), 15 g of Lewait M 600 (Bayer-Leverkusen) charged with NO\(_3\) were mixed with the sand in each culture tray, adding about 15 \(\mu\)mol of nitrogen to the substrate. The 200 seedlings of each tray did not seriously deplete nitrate from this, since nitrate reductase activity in the plants remained relatively constant during 2 weeks of growth (Fig. 1). The seedlings were kept for 10 days at 20 C under 200 ft-c continuous irradiation (40 w fluorescent tubes, Osram Fluora) and then for 4 days were transferred to growth chambers kept at 4, 10, 20 30, or 36 C. At the end of this temperature pretreatment the acclimated plants were harvested in batches of 20 and used for protein synthesis experiments. The rate of protein synthesis was measured at 4, 12.5, 20, 27.5, 35, 42.5, and 50 C to obtain Arrhenius curves.

Incubation with \(^{14}C\)-Leucine. Typically, 20 shoots were tied together loosely with a thread and submerged completely in the radioactive leucine solution (10\(^{-3}\) M, 0.166 \(\mu\)Ci/ml). Incubation time was 15 min unless otherwise noted. Incubation was performed in a U shaped vessel (Fig. 2, inset) held in a temperature-controlled water bath and illuminated with about 100 ft-c in-

\footnote{This work was supported by Deutsche Forschungsgemeinschaft.}
ance was disks) into the rated and then dry pretreatment temperature at min) and the supernatant tubes, and after stirring, leucine into other amino acids or incorporation into insoluble carbohydrate is remarkably small (15, 20). The lag phase for incorporation of leucine into protein is short (4 min in our experiments), since the metabolic pool of leucine is small compared to the total soluble pool (24). Incorporation rates of labeled precursor are nearly constant and independent of concentration within limits, namely, when high concentrations are used (i.e. 10⁻⁷ M leucine in our experiments) at which the specific activity of the metabolic pool approaches that of the added label (swamping; 12). However, to avoid possible metabolic disturbances resulting from such high concentrations of amino acids, incubation time should be minimized. A short incubation time should be adhered to also for the following reasons. First, the method of total immersion of the shoots into leucine solution is not physiologically realistic, in spite of the linearity obtained. Second, the seedlings should not be exposed to temperatures different from the temperature pretreatment for a time longer than absolutely necessary, otherwise their temperature adaptation might be endangered during the leucine incorporation experiments performed at temperatures from 5 to 50 C.

**Determination of Protein Content and Incorporated Activity.** Ethanol extraction of pigments and other solutes (including non-incorporated ¹⁴C-leucine) was carried out as follows. The ground material (frozen "dry" powder) was transferred into centrifuge tubes, and 40 ml of 80% ethanol were added to each sample. After stirring, the homogenate was centrifuged at 30,000g (15 min) and the supernatant discarded. This procedure was repeated until the supernatant was colorless. The pellet was treated with 5% trichloroacetic acid for 20 min at 80 C to solubilize nucleic acids and then rinsed once in a small Büchner funnel with cold trichloroacetic acid, 4 times with distilled H₂O, and twice with dry acetone (14).

For estimation of protein content and radioactivity incorporated into the protein fraction, the samples (including filter paper disks) were transferred back into centrifuge tubes, and 10 ml of Biuret reagent were added. After 3 hr, when maximum absorbance was reached, the supernatant was cleared by centrifugation, and absorbance was measured at 540 nm against a Biuret blank. Protein concentration was estimated from a standard curve, using purified wheat protein. The Biuret test was calibrated against the micro-Kjeldahl method (Fig. 3). An aliquot of the Biuret-protein solution was used directly for determination of radioactivity: 0.4 ml was mixed with 15 ml of scintillation liquid (12 g of butyl-PBD, 1 liter of toluene, 1 liter of methanol, both scintillation grade) and counted. The Biuret reagent did not interfere with the scintillation counting.

An analysis of variance and t test was performed for the regression lines of the Arrhenius plots of protein synthesis.

**Fig. 3. Calibration curve for determination of wheat protein.** Biuret reaction versus Kjeldahl assay of wheat-protein nitrogen. Inset: kinetics of color development of the Biuret reaction for three protein concentrations (mg/30 ml reagent).
RESULTS

Incorporation rate of $^{14}$C-leucine into the total protein fraction of the wheat shoots (dpm/mg protein) is nearly constant for at least 60 min following a lag phase of about 4 min (Fig. 2). In the following, all results are expressed in dpm/mg protein, since fresh weights were variable and dependent upon experimental conditions.

It was necessary to insure at the beginning that leucine uptake from external solution is not only linear but also does not itself affect the rate of protein synthesis of the seedlings. Hence an experiment was designed to check this: $^{14}$C for incorporation into protein was furnished via photosynthetically assimilated gaseous $^{14}$CO$_2$ to plants in contact either with nonradioactive leucine solution or with water. Shaking of the closed flasks insured constant saturation of the solution with HCO$_3^-$- Plants were in contact any time both with the solution and with the $^{14}$CO$_2$-containing atmosphere. Figure 4 shows that for at least 60 min the incorporation of assimilation products into protein is identical for plants immersed in water and in leucine, so external leucine does not influence protein synthesis. This indicates that any disruption of metabolic pathways associated with protein synthesis by high concentrations of $^{14}$C-leucine is unlikely to occur and that the temperature coefficient for leucine incorporation into protein does not reflect the temperature dependence of leucine uptake.

The Arrhenius plots for all five different temperature pretreatments are shown in Figure 5. The following three points are notable. First, the optimum temperature of protein synthesis shows a positive correlation with acclimation-temperature. The optimum temperature for seedlings kept at 4 or 10°C is close to 27.5°C; 35°C is the optimum temperature for plants pretreated at 36°C. For plants grown at medium temperatures (20 to 30°C) the op

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Fig. 4. Kinetics of incorporation of $^{14}$C-labeled assimilation products into the protein of wheat shoots in contact either with $^{14}$CO$_2$ and H$_2$O (●) or with $^{14}$CO$_2$ and leucine solution, $10^{-4}$M (■).

Fig. 5. Arrhenius plots of protein synthesis rates covering the temperature range 5°C to 50°C for wheat seedlings acclimated to 4, 10, 20, 30, and 36°C. The values for activation energy, computed from the regression lines, are inserted.
timum is somewhere near 32 °C. Second, the activation energy of protein synthesis is about 11 kcal/mole for seedlings grown at 4 to 20 °C, but higher growth temperatures cause the activation energy to increase to about 15 kcal/mole. This difference is statistically significant on the P = 0.001 level (Table I). Third, the decline of the rate of protein synthesis beyond the temperature-optimum is also modified by the pretreatment of the seedlings. Plants acclimated to 4 to 20 °C do not show a sharp decrease with rapidly increasing slope, generally typical for Arrhenius curves of enzymic reactions, but instead a linear and rather moderate decline as the high temperature region is approached. Pretreatment of the plants at 30 or 36 °C results in the "normal" shape of Arrhenius curves in the high temperature region.

At about 20 °C all Arrhenius curves intersect. At this temperature the rate of protein synthesis is the same for seedlings pretreated at low, medium, or elevated temperatures (standard deviation 13%). Figure 6 shows a linear plot of the temperature curves standardized on the mean of the 20 °C values, which depicts more clearly the absolute levels of protein synthesis. When 4 °C plants and 36 °C plants are compared, for example, protein synthesis at 35 °C is almost three times as high in plants "adapted" to elevated temperatures as in plants kept at low temperatures. Even plants grown at 30 °C have a rate of protein synthesis at 35 °C which is 220% of the rate for 4 °C-plants. On the other hand, at 5 °C, the rate of protein synthesis for plants pretreated at 4 °C amounts to twice the rate which 30 °C plants or 36 °C plants have at 4 °C. These differences at low temperatures are not statistically different as such, but the regression lines for the temperature range 5 to 27.5 °C are (Table I). The protein content of seedlings acclimated to 10 and 36 °C, respectively, differs only by about 17% on a dry weight basis (Fig. 6, inset). Hence the shifts of the optimum temperature of protein synthesis and the increase in activation energy bring about a substantial enhancement of protein turnover.

**DISCUSSION**

The Arrhenius curves for protein synthesis in wheat shoots closely resemble the Arrhenius curves for a single, enzyme-catalyzed reaction, except for the fact that with plants acclimated to 4 to 20 °C the decline beyond the temperature optimum is rather gradual. Arrhenius plots of other complex biological processes such as photosynthesis (2), bacterial growth (13), or oxidative respiration of mitochondria (21) may in certain cases also give a linear curve over a wide temperature range with a rather sharp inflection point when the optimum temperature is reached. The simplest, though not entirely precise, interpretation is that under such conditions the rate of the total process, consisting of several steps, is largely limited by the slowest reaction (5). Naturally, the Arrhenius curves of protein synthesis alone give no indication of what the rate-limiting reaction might be following a certain temperature pretreatment. The linearity of the ascending part of the curves favors the conclusion, however, that this reaction is directly involved in polypeptide formation, rather than being peripheral. It seems highly unrealistic to assume that a peripheral reaction would be able to impose its reaction rate and, hence, temperature characteristics on an entire metabolic network as complex as protein synthesis.

The acclimation of wheat seedlings to high growth temperatures can be explained in different ways. Several metabolic steps of protein synthesis may become rate-limiting successively as acclimation temperature rises. For example, specific metabolic pathways might be enhanced or suppressed as a result of temperature changes, because the temperature-dependence of Michaelis-Menten constants of certain key enzymes is different (7, 10, 30). Alternatively, only one enzymic reaction might limit the rate of protein synthesis over the whole range of acclimation temperatures, whereby the enzyme either gradually changes its temperature characteristics by undergoing alterations of its 3° and 4° structure (6, 18, 29) or it is active as one of several isozymes, each specifically suited for function in distinct thermal ranges (10).
Among the weak interactions determining the higher orders of protein structure, hydrophobic bonds and hydrogen bonds are most essential. Hydrophobic bonds are predominantly important for cold inactivation of enzyme molecules because their "free energy of denaturation" becomes negative at low temperature (4, 17, 19). Hydrogen bonding, on the other hand, becomes weakened at high temperature (11, 16, 27). It cannot be evaluated yet to which extent these two types of bonds contribute to heat acclimation, although we are tempted to concede hydrogen bonding a more important role because stimulation of protein synthesis at high temperatures must first of all counteract the breakdown of hydrogen bonds.

The linear curve of decline of protein synthesis activity beyond the optimum temperature obtained for plants that were acclimated to 4 to 20 °C does not seem to reflect protein denaturation. If not, then it is not one single denaturation process that determines the rate of protein synthesis in the high temperature region; rather, several reactions with different optimum temperatures become rate-limiting successively as higher temperatures are approached, each for only a small temperature range.

When adaptation is the ability to maintain a particular systemic state by compensating for environmentally induced irregularities in the flow of material and energy, then the adaptation of protein synthesis to changing temperature seems to be a "paradoxical adaptation" in the definition of Precht et al. (26)—characterized by acceleration instead of retardation of metabolic processes by high temperatures. This adaptive acceleration must not be confused with the inherent increase of reaction rates with rising temperature. Such inverse compensation is not infrequently reported for ectothermic animals, mostly for enzymes which are involved in degradative processes (8, 30, 31). In plant metabolism, however, it is photosynthesis which exhibits this adaptation pattern (22). Generally, inverse compensation is assumed to be participating in the mechanisms for homeostatic control of metabolism, being required to maintain metabolic balance, rather than to disturb it (30). Our experiments support this point of view: predominantly warm stimulation of protein synthesis is an expression of adaptive stimulation of protein turnover while protein concentration remains almost stationary.

It will require additional investigations to find out, if capacity adaptation of protein synthesis is a general phenomenon of plant metabolism, if qualitative changes accompany the rate-adaptation of protein synthesis and if conclusive evidence can be obtained that this effect plays an important role in heat resistance.

Acknowledgment—We thank Prof. F. B. Salisbury for critical reading of the manuscript.

LITERATURE CITED