Plant Physiol. (1987) 83, 497–499
0032-0889/87/83/0497/03/$01.00/0

Quantification of the Kinetin Effect on Protein Synthesis and Degradation in Senescing Wheat Leaves[1,2]

Received for publication October 10, 1986 and in revised form October 23, 1986

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ABSTRACT

Wheat leaves (*Triticum aestivum* L. cv San Agustin INTA) were detached when they reached maximum expansion, put individually in tubes containing water and left in darkness. After 3 days the protein content had decreased to 46% of the initial value. When the leaves were placed in 1 micromolar kinetin, they retained 60% of the initial protein content for the same period. This effect was observed only when leaves were treated with kinetin within the first 24 hours after detachment. The action of kinetin on both protein synthesis and degradation was quantitatively measured. Synthesis was estimated by the incorporation of L-[^{3}H]leucine into proteins. It was higher in kinetin treated than in non treated leaves. It contributed to about 14 micrograms of protein retention per leaf in 3 days. Measurement of protein degradation, evaluated by the decay of radioactivity in leaf proteins previously labeled with L-[^{3}H] leucine or as the difference between rates of protein synthesis and protein content, showed that kinetin decreased protein breakdown rates. It accounted for about 186 micrograms of protein retention per leaf in 3 days. Hence, kinetin action on protein breakdown was 13-fold average higher than its action on synthesis for the conservation of leaf protein. This difference is higher in early stages of the process.

Since the initial study of Richmond and Lang (12) in 1957, in which it was reported that kinetin retarded senescence of detached cocklebur leaves, many authors have confirmed this effect for different cytokinins on numerous species (3, 6–8). Detached organs treated with kinetin maintain a higher level of proteins, Chl and nucleic acids than controls. However, this synthetic growth regulator has little or no effect as a retardant of senescence in attached organs (9).

The decline in protein loss could be attributed to the involvement of kinetin either in the activation of protein synthesis, or in the inhibition of degradation. There are several pieces of evidence showing protein breakdown as the principal event affected by kinetin treatment (4, 15, 17, 18). At the same time, these authors and others (10, 13, 16, 19, 20) have observed that cytokinin treatment also stimulates or maintains the ability of isolated leaves to incorporate protein precursors. However, none of these studies yield a quantitative analysis of the extent by which the hormone affects the processes of protein synthesis and breakdown. The availability of new reliable methods for the measurements of protein synthesis and breakdown (5) makes a reexamination of the problem interesting. We report herein the quantitative data on kinetin effect on the rates of protein synthesis and degradation in the first leaf of wheat detached when they reached maximum expansion and left in darkness.

MATERIALS AND METHODS

Plant Material and Chemicals. Wheat (*Triticum aestivum* L. cv San Agustin INTA) was generously supplied by the Balcarce Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. Plants were grown as previously described (5). At the indicated times, leaves were transferred to kinetin solution. Kinetin and L-leucine were purchased from Sigma and L-[4,5-^{3}H]leucine from Amersham. All other reagents were of the highest purity available.

Analytical Procedures. Leaves were homogenized at 4°C in a glass homogenizer containing 40 μl of acetone per mg of leaf fresh weight. Acetone extracts containing Chl were separated by centrifugation at 2000*g* for 15 min. This extraction was repeated twice. The resulting cleared pellets were processed for the estimation of protein content according to Gornall *et al.* (2). Radioactivity in proteins was measured by scintillation spectrometry as described by Lamattina *et al.* (5). All results are presented as means ±SD.

Measurement of Rates of Protein Synthesis and Degradation. At the indicated times after detachment, leaves were labeled with a solution of 100 mM L-[^{3}H]leucine (357 μCi/mmol). Radioactivity in leaf proteins was determined 2 and 4 h after precursor addition and the rate of protein synthesis was calculated as previously described (5). Protein degradation rate was measured by labeling leaf protein in the moment of detachment with 4 μCi of L-[^{3}H]leucine (131 Ci/mmol) for 1 h, followed by a 4 h chase with 100 mM nonradioactive leucine to avoid recycling of labeled aminoacids. Measurement of radioactivity in proteins was performed as described (5).

RESULTS AND DISCUSSION

Fully expanded leaves (13 d old) were detached and put in darkness with water or different concentrations of kinetin. Their protein content was measured daily during a period of 3 d. Leaves treated with 1 μM kinetin retain 32% more protein after 3 d of detachment (200 μg protein per leaf, Table I, *P* column) than controls. This effect on the maintenance of leaf protein is in agreement with previous reports (18). One μM kinetin was

[1] Dedicated to Professor L. F. Leloir on the occasion of his 80th birthday.

[2] Partially supported by the Comisión de Investigaciones Científicas de la provincia de Buenos Aires (CIC), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, and the Alexander von Humboldt Stiftung, F.R.G.

[3] This paper is part of a dissertation of L. L. to be presented, in partial fulfillment of the requirements for the Degree of Doctor at the Universidad Nacional de Mar del Plata.

Table I. *Estimation of Protein Content and Rates of Protein Synthesis*

Leaves were cut and processed at the times indicated in the "Materials and Methods." The total protein content of whole leaf (P) was determined. Rates of synthesis for control and treated leaves (S) were estimated each day after detachment by equation $S = V/F_{leu}$ from the following data: (V) mass of leucine incorporated per leaf and per day and (F_{leu}) leucine proportion in leaf protein. The same F_{leu} was used for both control and kinetin treated leaves since no significant differences were detected between them. Each point represents at least three leaves and it is the average of five experiments.

Time after Detachment	Treatment	P	V	F_{leu}	S
h		μg	$\mu g\ Leu \cdot d^{-1}$	%	$\mu g \cdot d^{-1}$
0		1370 ± 100	2.32 ± 0.23	10.25 ± 0.5	22.7 ± 1.6
24	Control	1080 ± 54	2.07 ± 0.17	10.40 ± 1.2	19.9 ± 1.9
	Kinetin	1200 ± 60	2.50 ± 0.21		24.0 ± 2.3
48	Control	820 ± 80	1.82 ± 0.36	12.10 ± 2.1	15.0 ± 2.7
	Kinetin	960 ± 58	2.56 ± 0.50		21.1 ± 3.8
72	Control	630 ± 38	1.77 ± 0.38	10.35 ± 0.6	17.2 ± 3.2
	Kinetin	830 ± 41	2.32 ± 0.31		22.5 ± 2.1

Table II. *Estimation of Rates of Protein Breakdown for Control and Treated Leaves*

Values of synthesis at the initiation and at the end of each day were averaged. The net protein changes were calculated as the differences between protein content at the beginning and at the end of each period from Table I. Rates of protein degradation were obtained from the difference between synthesis and net protein change (degradation = $S - \Delta P$). Differences between kinetin and control values for both synthesis and degradation are indicated as Δ.

Interval after Detachment	Treatment	Protein Variation				
		Synthesis	Δ	Change	Degradation	Δ
				$\mu g \cdot d^{-1} \cdot leaf^{-1}$		
0–24	Control	21. ± 2	2	−290. ± 17	311. ± 21	−118
	Kinetin	23. ± 2		−170. ± 10	193. ± 13	
24–48	Control	17. ± 2	6	−260. ± 18	277. ± 28	−14
	Kinetin	23. ± 3		−240. ± 13	263. ± 24	
48–72	Control	16. ± 2	6	−190. ± 14	206. ± 23	−54
	Kinetin	22. ± 3		−130. ± 7	152. ± 14	

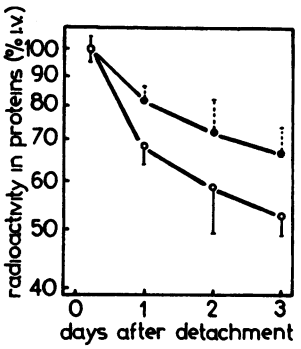

FIG. 1. Disappearance of protein radioactivity from leaves. Each leaf was labeled with [^{3}H]leucine immediately after its detachment and then chased with nonradioactive leucine. After that, they were transferred to 1 μM kinetin (●) or water (○). Five h after detachment the first point was taken and it represented 1600 cpm per leaf in average. Percent of initial value (%I.V.). Each point represents at least five leaves processed individually and bars, SD limits. Probability of significant differences between the kinetin-treated and control groups was calculated by the Student t test. Significance levels: 1 d, $P < 0.05$; 2 d, $P < 0.02$; 3 d, $P < 0.01$.

found to be the lowest, and nearest to the physiological concentration (11) at which significant differences were obtained for this system. All other experiments were performed at this concentration. To determine the ability of kinetin to maintain the level of protein at any moment after leaf detachment, leaves were detached, placed in darkness, and transferred to kinetin solutions 24, 48, and 72 h later. Results indicated that only when leaves were treated with kinetin within the first 24 h after detachment, was it possible to observe a delay in protein loss (data not shown). Similar results were also obtained by Stoddart and Thomas (14) with *Lolium temulentum* leaves.

The decrease of protein loss by kinetin could be due to an increase in the rates of protein synthesis. Experiments were performed to estimate the rate of protein synthesis (μg of protein synthesized per leaf and per day) at different times after detachment, in leaves put either in water or 1 μM kinetin. In these experiments, 100 μM leucine was used to expand the endogenous pool available for protein synthesis. This leucine concentration was previously found to be adequate and does not interfere with the incorporation of other amino acids (5). The rates of synthesis (S) were calculated from the μg of leucine incorporated per day into total leaf protein (V) and the leucine proportion in the leaf (F_{leu}) as previously described (5). Table I indicates estimations of S for leaves put in 1 μM kinetin or water (control) for several times after detachment. It shows that kinetin increased V by 21 to 41% over the control values. However, this effect produced by kinetin accounted for a small increase in the amount of protein synthesized (Table I, S column). When these increases are translated in terms of mass, they amount to 14 μg of protein in 72 h (Table II). Tavares and Kende (15) had also found that incorporation of radioactive leucine into proteins of cytokinin-treated leaves of *Zea mays* increased threefold over the control. They concluded that, in spite of this increased incorporation,

protein synthesis was not the main process involved in the retention of protein produced by kinetin. It should be pointed out that caution must be taken when evaluating only the incorporation of radioactivity. It could be helpful to obtain evidences on the relative magnitude of some processes, like comparison between protein synthesis rates of control and kinetin-treated leaves. However, no further interpretation of these data is possible.

The rates of degradation (μg of protein degraded per leaf and per day) were indirectly calculated as the difference between synthesis (S) and the protein change (ΔP). Table II shows that kinetin inhibits degradation, resulting in a protein retention of about 118 μg in the first 24 h and 68 μg thereafter. Another experiment was performed to confirm the above described indirect estimations of protein degradation. Immediately after detachment, leaves were pulsed for 60 min with L-[^{3}H]leucine of high specific activity and then chased with nonradioactive leucine. After chase, leaves were put in darkness in solutions containing kinetin or water. Radioactivity in proteins was measured during the following 3 d. Figure 1 indicates that kinetin-treated leaves retained about 30% more label than controls after 3 d of detachment. These results indicate that there is a close correlation between direct and indirect methods for estimation of protein degradation.

Hence, the extent of kinetin effect on protein degradation is at least 13-fold higher than its effect on protein synthesis in a 72 h period. This strongly supports the concept that kinetin action is exerted by diminishing the protein degradation rate, previously postulated by other authors (13, 15, 18).

In plant cells, as in other systems (bacteria and animal cells), protein breakdown is important in determining developmental and adaptative phenomena (1). This work is an example of how the rate of degradation of cell proteins is determined by the hormonal status of the cell. Variations in degradation rates should be a quick and primary strategy of cells under stress conditions to regulate their protein mass.

Acknowledgments—We are indebted to our colleagues in the Instituto de Investigaciones Biológicas for continuous help and criticism. L. L. holds a postgraduate fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), R. D. C. is an established investigator of CONICET, and R. P. L. is a career investigator of Comisión de Investigaciones Científicas.

LITERATURE CITED

1. DAVIES DD 1982 Physiological aspects of protein turnover. *In* D Boulter, B Parthier, eds, Encyclopedia of Plant Physiology, Vol 14A, Nucleic Acids and Proteins in Plants. Springer-Verlag, Berlin, pp 189–219
2. GORNALL AC, CJ BARDAWILL, MM DAVID 1949 Determination of serum proteins by means of the Biuret reaction. J Biol Chem 177:751–766
3. KULAEVA ON 1962 The effect of roots on leaf metabolism in relation to the action of kinetin on leaves. Fiziol Rast 9:182–189
4. KURAISHI S 1968 The effect of kinetin on protein level of *Brassica* leaf disks. Physiol Plant 21:78–83
5. LAMATTINA L, R PONT LEZICA, RD CONDE 1985 Protein metabolism in senescing wheat leaves. Determination of synthesis and degradation rates and their effects on protein loss. Plant Physiol 77:587–590
6. MOORE AE, BA STONE 1970 Effect of senescence and hormone treatment on the β-1,3-glucan hydrolase in *Nicotiana glutinosa* leaves. *In* DJ Carr, ed, Plant Growth Substances. Springer-Verlag, Berlin, pp 598–603
7. MOTHES K, AC 1960 Über das Altern der Blätter und die Möglichkeit ihrer Wiederverjungung. Naturwissenschaften 47:335–337
8. MULLER K, AC LEOPOLD 1966 Correlative aging and transport of ^{32}P in corn leaves under the influence of kinetin. Planta 68:167–185
9. NOODEN LD, AC LEOPOLD 1978 Phytohormones and the endogenous regulation of senescence and abscission. *In* DS Letham, TW Goodwin, TJ Higgins, eds, Phytohormones and Related Compounds. A comprehensive Treatise, Vol II. Elsevier/North-Holland, Amsterdam, pp 329–369
10. OSBORNE DJ 1962 Effect of kinetin on protein and nucleic acids metabolism in *Xanthium* leaves during senescence. Plant Physiol 37: 595–602
11. OSBORNE DJ 1974 Hormones and the shedding of leaves and bolls. Cotton Grow Rev 51:256–265
12. RICHMOND AE, A LANG 1957 Effect of kinetin on protein content and survival of detached *Xanthium* leaves. Science 125:650–651
13. SHIBAOKA H, KV THIMANN 1970 Antagonisms between kinetin and amino acids. Experiments on the mode of action of cytokinins. Plant Physiol 46:212–220
14. STODDART JL, H THOMAS 1982 Leaf senescence. *In* D Boulter, B Parthier, eds, Encyclopedia of Plant Physiology, Vol 14A, Nucleic Acids and Proteins in Plants. Springer-Verlag, Berlin, pp 592–626
15. TAVARES J, H KENDE 1970 The effect of 6-benzylaminopurine on protein metabolism in senescing wheat leaves. Phytochemistry 9:1763–1770
16. TEPFER DA, DE FOSKETT 1978 Hormone-mediated translational control of protein synthesis in cultured cells of *Glycine max*. Dev Biol 62:486–492
17. TREWAVAS A 1972 Control of the protein turnover rates in *Lemna minor*. Plant Physiol 49:47–51
18. TUNG HF, CJ BRADY 1970 Kinetin treatment and protein synthesis in detached wheat leaves. *In* DJ Carr, ed, Plant Growth Substances. Springer-Verlag, Berlin, pp 589–597
19. WOLLGIEHN R 1961 Untersuchungen über den Einfluss des Kinetins auf den Nucleinsaure-und Protein stoffwechsel isolierter Blätter. Flora 151:411–437
20. WOOLHOUSE HW, GI JENKINS 1983 Physiological responses, metabolic changes and regulation during leaf senescence. *In* FL Milthorp, J Dale, eds, The Growth and Functioning of Leaves. University Press, Cambridge, pp 449–487