Activity of Aminoacyl-Transfer-Ribonucleic Acid Synthetases in Tobacco-Leaf Tissue in Relation to Senescence and to the Action of 6-Furfurylaminopurine

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1. The absolute aminoacyl-transfer-RNA synthetase activity decreased with increasing physiological age of the tobacco leaf, but the specific activity did not decrease until the chlorophyll content fell below about 20% of that in the young leaf. 2. In leaf disks floated on water, the absolute aminoacyl-transfer-RNA synthetase activity increased after 2 days but then decreased until the end of the experiment (12 days). Since the concentration of soluble protein decreased, the specific activity increased throughout the experiment. 3. The absolute and specific aminoacyl-transfer-RNA synthetase activities increased above control values in disks treated with 6-furfurylaminopurine (kinetin) after 4 and 6 days respectively, but the effect was too slow to account for the delay in decrease of chlorophyll and the delay in increase of α -amino nitrogen induced by kinetin. 4. After treatment for 7 days, the absolute aminoacyl-transfer-RNA synthetase activity increased in disks treated with kinetin between 0.5 and 1.6 μ M but the concentration of α -amino nitrogen decreased with kinetin concentrations between 0.05 and 50 μ M.

The concentration of soluble protein per unit area of tobacco leaf decreases with increasing physiological age. Since the absolute peptidase activity per unit area remains constant, Anderson & Rowan (1965) concluded that stability of peptidase is an important factor in senescence of leaves and that rate of protein synthesis regulated the concentration of protein in the leaf.

When leaf disks are treated with 6-furfurylaminopurine (kinetin), an inhibitor of senescence in intact leaves and leaf disks (Richmond & Lang, 1957; Osborne, 1962; Sugiura, Umemura & Oota, 1962), the concentration of α -amino nitrogen decreases within 24 hr. (Anderson & Rowan, 1966a). Kinetin does not delay senescence by inhibiting peptidase activity, since this effect is not significant in disks treated for less than 4 days. Anderson & Rowan (1966a) concluded that kinetin acts by stimulating synthesis of protein rather than by inhibiting degradation, confirming the conclusions drawn by Osborne (1962) from studies of the effect of kinetin on the incorporation of L-[1- 14 C]leucine into protein in leaves of *Xanthium pennsylvanicum* Wall.

In the present paper we have examined the hypothesis that the aminoacyl-s-RNA $\dagger$ synthetase

activity regulates the rate of protein synthesis in senescent leaves and that retention of activity of these enzymes underlies the delay of senescence induced in leaf disks by kinetin.

MATERIALS AND METHODS

Disks of tobacco leaf were prepared as described by Anderson & Rowan (1966b) and chlorophyll and α -amino N were estimated by the method of Anderson & Rowan (1965). The chlorophyll in each sample was extracted in 25 ml. of 80% (v/v) ethanol. Soluble protein and aminoacyl-s-RNA synthetase activity were estimated as described by Anderson & Rowan (1966b). Leaf disks were extracted with 1 ml. of extraction medium/5 disks and the synthetase activity was assayed at 0.8 disk/ml. of extract. Absolute synthetase activity is expressed as units/ml. of undiluted extract (5 disks/ml.). Leaf disks were treated with kinetin by the method of Anderson & Rowan (1966a). L-Amino acids and amides were supplied by Mann Research Laboratories Inc., New York, N.Y., U.S.A. Glycine was supplied by British Drug Houses Ltd., Poole, Dorset. Kinetin was supplied by Sigma Chemical Co., St Louis, Mo., U.S.A. When required, the least significant difference between two mean values was calculated by a *t* test after analysis of variance.

RESULTS

Changes in the aminoacyl-s-RNA synthetase activity with physiological age of leaf. Samples of disks were prepared from a series of leaves of a single plant and the concentrations of α -amino

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$\dagger$ Abbreviation: s-RNA, transfer or 'soluble' RNA.

nitrogen, chlorophyll and soluble protein and absolute and specific synthetase activities were measured (Fig. 1). The concentrations of α -amino nitrogen, chlorophyll and soluble protein decreased with increasing physiological age of leaf, confirming previous results (Anderson & Rowan, 1965). The absolute synthetase activity per unit area decreased with increasing physiological age of leaf. Thus activity was very low in the lower, yellow, leaves. The specific activity was virtually constant in all except the oldest leaf in which specific activity was significantly lower than in upper leaves.

Changes in the aminoacyl-s-RNA synthetase activity in leaf disks after harvesting. The absolute activity of disks floated on water increased during the first 2 days and then decreased (Fig. 2). Throughout the experiment (12 days) the con-

centration of soluble protein decreased, giving rise to an increase in specific synthetase activity; the concentration of chlorophyll decreased, whereas that of α -amino nitrogen increased, confirming the observations of Anderson & Rowan (1965).

The extent of the initial increase in activity after 2 days varied but it was found in all experiments. As this could be due to decreased retention of the synthetases on particles in the preparations prepared from disks floated on water for 2 days, the effect of ultrasonic treatment on extracts prepared from disks cut from freshly prepared leaves was examined. Leaf disks were ground in the usual manner and the resulting slurry was transferred to centrifuge tubes. These were placed for 3 min. in the bath of a unit (Ultrasonic Industries Inc., model C40C2PK-15 coupled to a model T-40C1 transducer) producing 80kc/sec., and

Fig. 1. Changes in absolute (●) and specific (○) aminoacyl-s-RNA synthetase activities and in concentrations of α -amino N (▲), chlorophyll (△) and soluble protein (■) with increase in physiological age of leaf. All leaves were taken from a single plant. Samples containing 35 disks were ground in 7.0 ml. of extraction medium.

Fig. 2. Effect of kinetin on changes in concentrations of α -amino N, chlorophyll and soluble protein and in absolute and specific aminoacyl-s-RNA synthetase activities in leaf disks with time after harvest. Samples containing 20 disks were incubated on water (●) or 50 μ M-kinetin (○) and ground in 4.0 ml. of extraction medium. Vertical lines show the least significant difference (P 0.05) between two mean values. All results are means of duplicate extractions.

the supernatant fraction was recovered by centrifugation. Ultrasonic treatment did not increase either absolute or specific synthetase activity and we conclude that decreased retention of the enzymes on particles does not explain the higher activity found in extracts from disks floated on water for 2 days.

Effect of duration of treatment of leaf disks with kinetin on the aminoacyl-s-RNA synthetase activity. The absolute activity of the synthetases extracted from disks treated with kinetin ($50\mu\text{M}$) increased during the first 2 days (as in disks floated on water) and remained constant until the end of the experiment (Fig. 2). The activity of the enzymes extracted from disks floated on water decreased significantly below the activity extracted from disks treated with kinetin only after 4 days or more. The specific activity of the synthetases extracted from disks treated with kinetin increased throughout the experiment. The concentration of α -amino nitrogen decreased in disks treated with kinetin ($50\mu\text{M}$) and the difference in α -amino nitrogen between disks floated on water and

kinetin was significant after 2 days, confirming the results of Anderson & Rowan (1966a). Kinetin had no effect on the amount of protein extracted at pH 7.2.

Effect of kinetin concentration on the activity of aminoacyl-s-RNA synthetases extracted from leaf disks treated for 7 days. The absolute activity of synthetases extracted from leaf disks treated with kinetin at concentrations of $1.6\mu\text{M}$ and above was 40% higher than that in disks treated with $0.05\mu\text{M}$ kinetin, and 20% higher than in disks at day 0 (Fig. 3). Thus absolute synthetase activity increased with concentration of kinetin only between 0.5 and $1.6\mu\text{M}$ whereas the concentration of α -amino nitrogen decreased with kinetin between 0.05 and $50\mu\text{M}$. Thus concentration of α -amino nitrogen changes independently of changes in synthetase activity, confirming the results of the time study (Fig. 2).

DISCUSSION

Since the absolute peptidase activity does not change with age of leaf (Anderson & Rowan, 1965), decreasing absolute aminoacyl-s-RNA synthetase activity with increasing physiological age of the tobacco leaf is consistent with the regulation of protein synthesis by the synthetases in the attached leaf (Fig. 1). Alternatively, the decreasing absolute synthetase activity in the aging leaf may merely reflect the decreasing concentration of protein. Larger cell size does not account for the low absolute activity in the older leaves, since the cell volume is only slightly greater in old than in young leaves (Anderson & Rowan, 1965), whereas synthetase activity in young leaves is 13 times that in old leaves.

Henshall & Goodwin (1964) have suggested that increased activity of aminoacyl-s-RNA synthetases extracted from germinating pea seed after treatment with gibberellic acid was induced by the increase in concentration of amino acids resulting from this treatment. The correlation between decreasing synthetase activity of tobacco leaf with concentration of α -amino nitrogen (Fig. 1) is consistent with this hypothesis. However, this correlation does not occur in leaf disks treated with kinetin (Figs. 2 and 3) and thus the mechanism for the regulation of synthetase activity proposed by Henshall & Goodwin (1964) does not operate in leaf disks treated with kinetin.

In leaf disks, α -amino nitrogen cannot be removed by translocation and we have concluded that kinetin delays senescence by stimulating protein synthesis, which is reflected in a decrease in the concentration of α -amino nitrogen (Anderson & Rowan, 1966a). Aminoacyl-s-RNA synthetase activity increases significantly above control levels

Fig. 3. Effect of kinetin concentration after incubation for 7 days on changes in the concentrations of α -amino N (▲) and of chlorophyll (Δ) and in absolute (●) and specific (○) aminoacyl-s-RNA synthetase activities. The arrows show the concentration of α -amino N and absolute aminoacyl-s-RNA synthetase activity at harvest. Samples containing 20 disks were ground in 4.0 ml. of extraction medium.

only when leaf disks are treated with kinetin for at least 4 days (Figs. 2 and 3), but the concentration of α -amino nitrogen is significantly lower after 1 or 2 days (Anderson & Rowan, 1966a; Fig. 2). Also, changes in α -amino nitrogen induced by a range of concentrations of kinetin are independent of changes in synthetase activity (Fig. 3). Therefore, as decreases in α -amino nitrogen induced by treating with kinetin are independent of changes in synthetase activity, the primary action of kinetin in delaying senescence is not to maintain the activity of these enzymes.

Srivastava (1965) suggested that, as kinetin apparently inhibited ribonuclease activity in barley leaves (Srivastava & Ware, 1965), kinetin delayed senescence by preventing degradation of a fraction of RNA concerned in protein synthesis. However, the decrease in ribonuclease activity after 2 days was only 15% greater than in control tissue. Since we have found that the fresh weight of disks of tobacco leaf treated with kinetin was 12–17% higher than control disks, the ribonuclease activity is unlikely to have decreased in barley leaf treated with kinetin for 2 days, by which time kinetin clearly retarded senescence. Similarly, the apparent decrease in deoxyribonuclease activity reported by Srivastava & Ware (1965) in barley leaves is unlikely to have been significant after treating with kinetin for 2 days. Kinetin delays senescence in leaf tissue rapidly, and although changes in the activities of several enzymes have been reported in treated leaf tissue in addition to the present study (Udvardy, Horváth, Kisbán, Dézsi & Farkas, 1964; Srivastava & Ware, 1965; Anderson & Rowan, 1966a) the changes were not rapid enough to underlie the mode of action of kinetin.

Osborne (1962) has shown that the RNA/DNA ratio decreases during senescence of detached leaves and she suggested that the decrease in protein content could be due to a decrease in the sites of protein synthesis, rather than a decrease in the activities of enzymes concerned in protein synthesis. The high aminoacyl-s-RNA synthetase activity during senescence of leaf disks floated on water for 12 days (Fig. 2) is consistent with this hypothesis but the decreasing activity during senescence of attached leaves (Fig. 1) is not.

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REFERENCES

- Anderson, J. W. & Rowan, K. S. (1965). *Biochem. J.* **97**, 741.
Anderson, J. W. & Rowan, K. S. (1966a). *Biochem. J.* **98**, 401.
Anderson, J. W. & Rowan, K. S. (1966b). *Biochem. J.* **101**, 9.
Henshall, J. D. & Goodwin, T. W. (1964). *Phytochemistry*, **3**, 677.
Osborne, D. J. (1962). *Plant Physiol.* **37**, 595.
Richmond, A. E. & Lang, A. (1957). *Science*, **125**, 650.
Srivastava, B. I. S. (1965). *Arch. Biochem. Biophys.* **110**, 97.
Srivastava, B. I. S. & Ware, G. (1965). *Plant Physiol.* **40**, 62.
Sugiura, M., Umemura, K. & Oota, Y. (1962). *Physiol. Plant.* **15**, 457.
Udvardy, J., Horváth, M., Kisbán, K., Dézsi, L. & Farkas, G. L. (1964). *Experientia*, **20**, 215.