Partial Inhibition of the Decay of Hill Activity in Isolated Chloroplasts by Kinetin

D. Banerji and N. Kumar

Institute of Advanced Studies, Meerut University, Meerut.

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Abstract.-

Kinetin when included in the grinding medium inhibits degeneration of isolated spinach chloroplasts. This is indicated by the relatively greater retention of Hill activity by chloroplasts isolated in the presence of Kinetin. It appears that the Kinetin effect is probably on the integrity of the chloroplasts membrane. The technique seems to be of great practical value in photosynthetic studies.

Kinetin was shown to be an inhibitor of leaf senescene[1, 2] and this effect has since been confirmed by various workers[3, 4, 5, 6] Isolated Chloroplasts also undergo rapid degradation during experimentation thus, creating a major problem for the investigator. It was therefore, of interest to see whether this ageing of chloroplasts can be checked by Kinetin *in Vitro* or not. Some of the *in vivo* effects of Kinetin[7] and GA_3 on nuclei[8] could be obtained *in vitro* by the inclusion of respective hormones in the grinding media for isolation. A similar approach with chloroplasts of radish leaves, met with success and it was observed that whereas chloroplasts isolated in grinding media without kinetin lost Ca. 77% of the initial chlorophyll in 14 h, those isolated in grinding media with kinetin lost Ca. 50% of the initial chlorophyll[9]. In the present paper, the effect of

inclusion of kinetin in the grinding medium, on the decline in Hill activity of isolated spinach chloroplasts is presented.

Chloroplasts were isolated at ca $4^{o}C$ by homogenising 5 g spinach leaves in 20 ml. of the grinding medium, (0.35 M NaCl, 0.05 M Tris HCl, pH 8.0 plus or minus 10 mg/l Kinetin), filtering through four layers of cheese cloth and one layer of Whatman No. 1 filter paper, centrifuging the filtrate at 100xg for 1 min and the resulting supernatant at 1000 x g for 5 min. The pellet was washed once by suspending and recentrifuging in a washing medium consisting of 0.175 M Nacl, 0.1 M K_2HPO_4 - KH_2PO_4 buffer pH 6.5. The washed pellet was suspended in the washing medium and made to 5 ml (Suspension A). In this manner two sets of chloroplasts suspensions 'A' were obtained, one of chloroplasts isolated with Kinetin in the grinding medium (plus Kinetin set) and another control set of chloroplasts isolated without kinetin in the grinding medium.

For following the rate of Hill activity, in two sets, 1 ml aliquots of each were made to 50 ml with the washing medium and the suspension (B) left at 20 + $2^{o}C$ under an incandescent 100 W bulb. 5 ml samples were taken at 0, 60, 150 min, mixed with 3.5×10^{-3}M DCPIP (2, 6 dichlorophenol indo-phenol), exposed to light from two incandescent 100 W bulbs (through a water jacket) for 5 min and the OD of these and dark controls read at 620 nm in Spectronic 20.

To determine the accompanying loss of chlorophyll, 0.1 ml aliquots of suspension A were made to 1 ml with washing medium, exposed to the same condition as the sets for Hill activity and the samples taken again at 0, 60, 150 min. Chlorophyll content was determined by shaking each suspension with 5 ml. acetone, centrifuging at 1000 x g for 5 min reading the O.D. of the extract at 652 nm to calculate total

chlorophyll[10]. As, 5 ml of suspension B was equivalent to 0.1 ml of suspension A. These values of chlorophyll were used for calculating the Hill activity in uM DCPIP/ mg chlorophyll / min.

The results are presented in Table 1 and represent the average of six experiments done in duplicate.

The data indicate that the decline in the amount of Chlorophyll upto 150 min is about the same, ca 71% of the initial, in the chloroplasts isolated either with or without Kinetin in the grinding medium. In Chloroplasts from radish leaves (8) the chlorophyll analysis was done at 14 h and at this time the amount of chlorophyll was much higher in those isolated in the presence of Kinetin than those without the hormone. In the short period of 150 min such difference in chlorophyll content, between the two sets does not become apparent. However, the table shows that the Kinetin effect is clearly observed on the Hill activity. Inspite of the fact that the amount of chlorophyll is same in the two sets, the Hill activity is higher in the chloroplasts of the plus Kinetin set than those isolated without Kinetin in the grinding medium. In the

Table 1.- Effect of inclusion of Kinetin in the grinding medium, on the decay of Hill activity of isolated Spinach chloroplasts.

Time	mg chlorophyll/5ml suspension		Hill activity uM DCPIP converted/mg Chl/min.	
	Minus Kinetin	Plus Kinetin	Minus Kinetin	Plus Kinetin
Initial	1.62	1.86	0.040	0.040
60 min	1.33	1.62	0.037	0.040
150 min	1.16	1.45	0.012	0.021

minus Kinetin, control set, Hill activity is 92% and 30% of the initial rate at 60 and 150 min respectively whereas in the plus Kinetin set the activity is same as initial at 60 min and 50% of the initial at 150 min. Inclusion of Kinetin in the grinding medium, thus seems to prevent degeneration of isolated chloroplasts. The comparatively low Hill activity obtained in these experiments seems to be due to the use of a light source of lower intensity. The chloroplasts isolated in the presence of Kinetin, seem to be of the unstripped type under the light microscope and Kinetin may have something to do with membrane integrity. However, a positive statement is not possible without further investigation. In any case, the previous report and the present one indicate that inclusion of Kinetin in the grinding medium, provides a technique of definite advantage, in experiments involving isolated chloroplasts. Further work, especially with leaves in which senescence is retarded with hormones other than Kinetin is in progress.

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