Pakistan J. Sci. Ind. Res., Vol. 13, No. 4, December 1970

INCORPORATION OF IAA TO PROTEINS AND RNA IN VITRO*

M.I. KHAN

Department of Botany, University of Karachi, Karachi 32

(Received July 4, 1969; revised March 30 1970)

The incorporation of IAA-1-^{14}C and IAA-2-^{14}C into buffer-soluble protein and RNA fractions of *Taraxacum* roots was studied *in vitro*. It was observed that a small proportion of the total auxin supplied was incorporated into protein and RNA. The incorporation of IAA-1-^{14}C increased linearly from 2–4 hr.

In the past attempts have been made to explore the physiological significant of bound auxins, auxin-complexes and auxin precursors in plant tissues.[1–6] Siegel and Galston[7] observed the coupling of IAA with pea root proteins *in vivo* and *in vitro*. Galston[8] found that the binding of IAA to the proteins of pea roots occurs only under conditions of active aerobic respiration.

The present work was designed to investigate the auxin (IAA) binding capacity of proteins and nucleic acid (RNA) in the crude homogenate of *Taraxacum* root *in vitro*.

Material and Methods

Taraxacum officinale Web. roots were extracted with 0.02M phosphate buffer, pH 7.4, and centrifuged in the cold (5°C) at 4000×g for 20 min. The supernatant solution, containing the buffer-soluble protein and RNA, was used for the study. The auxin used was IAA-1-^{14}C (specific activity= 183 μCi/mg) and IAA-2-^{14}C (specific activity= 16.1 μCi/mg), obtained from the Radiochemical Centre, Amersham, England.

IAA was added to 4 ml of the buffer-soluble extract (containing 1.8 mg of total protein and 0.61 mg of total RNA) to give a final concentration of 10^{-5}M. The tubes were kept in beakers covered with black-polythene sheets to prevent IAA destruction by light. These mixtures were incubated for 1–4 hr on a rotary shaker at 25°C in the dark. 5 ml of 10% trichloroacetic acid (T.C.A.) was then added to stop the reaction and the tubes were left at 5°C for 1 hr, after which protein was separated by centrifugation at 4000×g for 20 min. The precipitates were washed once with 5% TCA and three times with 20 ml of 95% ethanol. The supernatant solutions were pooled and the pH adjusted to 5 with 1N NaOH. A few drops of 10% NaCl were added and the solutions were allowed to stand overnight at 5°C. The RNA was then centrifuged down at 10,000×g for 20 min.[9]

Protein and RNA precipitates so obtained were washed with ether twice, to ensure that no IAA remained. The precipitates were dissolved separately in 2 ml of 0.5N NaOH and digested at 37°C for 4 hr. Their radioactivity was measured using a liquid-scintillation counter (I.D.L. type 6012), 0.2 ml aliquots of the solution being mixed with 3.0 ml of the liquid scintillator NE 220, (obtained from Nuclear Enterprises (G.B) Limited, Edinburgh) in 10 ml glass bottles. Each sample was counted for 400 sec.

Results and Discussion

Results of IAA incorporation into buffer-soluble proteins and RNA after 4 hr of incubation are presented in Table 1. It is clear from these results that a small proportion of IAA is incorporated into the protein and RNA fractions. An average of 1.36% IAA-2-^{14}C and 1.45% IAA-1-^{14}C of the total counts added was incorporated into buffer-soluble proteins while the percentage of total radioactivity in the buffer-soluble RNA was 0.742 in IAA-2-^{14}C and 0.644 in IAA-1-^{14}C treatments.

Kinetics of IAA-1-^{14}C incorporation into buffer-soluble proteins and RNA after 1,2,3 and 4 hr of incubation at 25°C are presented in Table 2 and Fig. 1. From these results it appears that a small proportion of the total auxin supplied is incorporated into protein and RNA after 1 hr and the rates of incorporation increases linearly from 2 to 4 hr (Fig. 1) in both the cases. The greater increase of IAA incorporation into protein and RNA from 1–2 hr might suggest the availability of more binding sites for IAA into these molecules than at later stages. The mechanism of IAA incorporation into protein and RNA fractions was not studied.

It is not clear from these results whether IAA or some metabolite of IAA was incorporated into

*This work was carried out at the Department of Botany, University of Sheffield, and is based on part of a thesis accepted for the degree of Ph. D.

TABLE 1.—IAA-2-^{14}C AND IAA-1-^{14}C INCORPORATION INTO BUFFER SOLUBLE PROTEINS AND RNA *in vitro*.

Replicate No.	IAA	Added cts/100 sec	cts/100 sec in total proteins recovered	% of total activity in proteins	cts/100 sec in total RNA recovered	% of total activity in RNA
I	2-^{14}C-	34,160	460	1.34	225	0.658
II	2-^{14}C-	34,160	450	1.31	256	0.749
III	2-^{14}C-	34,160	496	1.45	280	0.819
I	1-^{14}C-	226,060	3,154	1.39	1,465	0.648
II	1-^{14}C-	226,060	3,430	1.51	1,450	0.641
III	1-^{14}C-	226,060	3,290	1.45	1,460	0.645

TABLE 2.—KINETICS OF IAA-1-^{14}C INCORPORATION INTO BUFFER-SOLUBLE PROTEIN AND RNA. (Each value is an average of three replications)

Time hr	Added total cts/100 sec	total cts/100 sec recovered in total proteins	% total cts in proteins	total cts/100 sec recovered in total RNA	% total cts in RNA
1	226,030	800	0.35	180	0.07
2	226,030	2140	0.94	750	0.33
3	226,030	2560	1.13	1075	0.47
4	226,030	2988	1.32	1383	0.61

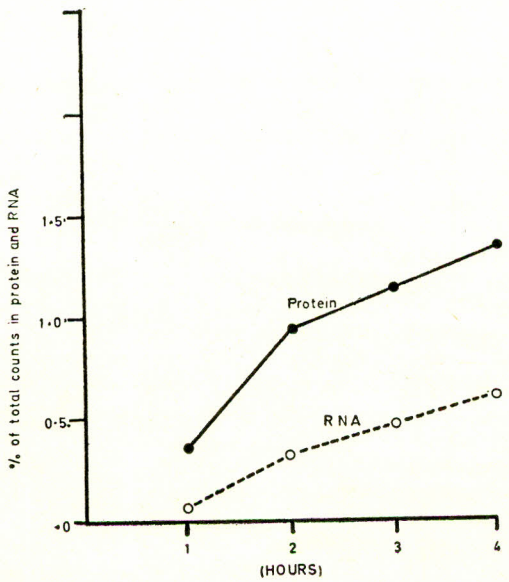

Fig. 1.—Kinetics of IAA-1-^{14}C incorporation into buffer soluble portion and RNA.

these fractions and further work was carried out to investigate this problem (details to be published elsewhere). RNA extract containing the radioactivity (IAA-2-^{14}C) was hydrolysed with 0.3 M KOH according to the technique of Davidson and Smellie.[10] Hydrolysed RNA yielded a radioactive spot at an *Rf* similar to that given by marker spot of IAA-2-^{14}C. However, hydrolysis of buffer-soluble proteins with proteolytic enzymes, acid and alkali did not release any free IAA suggesting that IAA, either in the form of IAA or in some other form is firmly attached with the proteins. Similar results were obtained by Siegel and Galston[7] using excised pea root tips.

Acknowledgement.—The author wishes to thank Dr. A. Booth for taking keen interest in the work.

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