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Short Communication

Nature of a Rapidly Labeled RNA Fraction Described in Higher Plant Systems

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When seedling tissues are exposed to $^{32}PO_4$ for short periods a rapidly labeled RNA fraction is frequently observed which is eluted from MAK columns as peak I between the 2 components of the so-called soluble RNA, e.g. in peanut cotyledons (5), in soybean hypocotyls (11), and in different parts of pea and bean seedlings (9). This ^{32}P-labeled fraction is characterized by a high GMP-CMP content. The radioactivity peak of the DNA-RNA fraction which preceeds the optical density profile is also rich in GMP-CMP (4, 9, 17) whereas the overall base composition of the DNA-RNA fraction has a low GMP-CMP content.

Several authors have been aware of the possibility that bacterial contamination could influence the results. By actual bacterial counts (19) and by the use of inhibitors (9, 11) it has been deduced that the microorganisms do not play a major role in producing the observed labeling pattern. It is, however, striking that in experiments with aseptic higher plant systems (13, 15,) there is a perfect matching of the radioactivity and the OD of the DNA-RNA peak.

In watermelon seedlings (*Citrullus vulgaris* Schrad.) we find only 4 species of Pseudomonas (kindly identified by Dr. Neidhardt's group) as bacterial contaminants. To check the effect of bacterial contamination upon the $^{32}PO_4$ incorporation into nucleic acids by the cotyledons, a bacteria-free system of watermelon seedlings was first obtained. During the exposure to $^{32}PO_4$, a culture of the bacteria mentioned above was added back to the incubation medium. The labeling of the nucleic acids in the washed cotyledons was then compared to that in the bacteria-free control. It is shown that both the labeled fractions mentioned above appeared only in the contaminated samples.

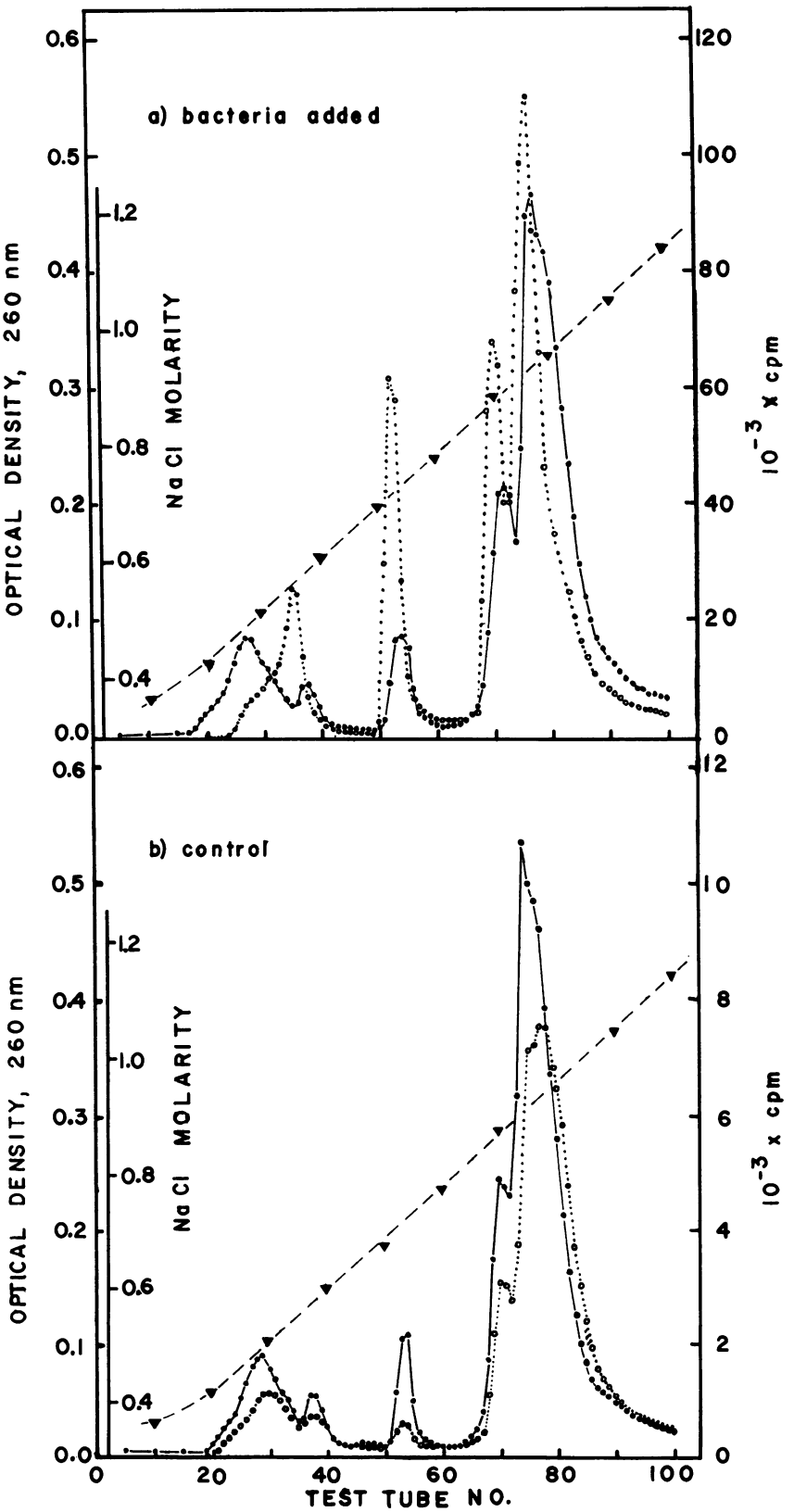
Methods

Seeds without testae were surface sterilized with 50 % Clorox (= 3 % NaOCl) for 5 minutes. This treatment reduces the growth of the seedlings after 5 days by 19 % compared to untreated controls; no other adverse effects were noticed. The sterilized seeds were then transferred to sterile boxes containing vermiculite and grown in the dark at 30°. After 1 and one-half days samples of 40 cotyledons were removed in dim green light (λ_{max} = 515 nm) and shaken in the dark for 3 hours at 30° in a sterile solution containing 6 ml 0.01 M tris, pH 6.7 and 0.5 mc $^{32}PO_4$. To 1 sample of 40 cotyledons bacteria cultured from normal watermelon seedlings were added. After the 3 hours incubation the bacterial count was 10^9 per 6 ml. The cotyledons were then removed from the medium and washed vigorously first with cold phosphate solution (0.01 M, pH 6.7) and then with distilled water. Only bacteria adhering to the surface of the cotyledons after this washing contributed to the results. The nucleic acids were extracted by the phenol method of Gierer and Schramm (8). Bentonite was used as ribonuclease inhibitor.

The nucleic acids were separated on MAK-columns as described by Mandell and Hershey (14) using a linear NaCl gradient ranging from 0.35 M to 1.20 M in 0.01 M tris, pH 6.9. Samples of 5 ml were collected. The OD was determined at 260 nm with a Beckman DU spectrophotometer and the radioactivity was determined with a Tracerlab gasflow counter. The NaCl concentration was measured with a Bausch and Lomb refractometer.

Results

As already pointed out by some authors (1, 16) bacterial contamination may contribute heavily to the phosphate uptake of higher plants, an effect which can not be overcome by thorough washing. A comparison of the right hand scale of figure 1a and 1b shows that the incorporation into the contaminated cotyledons was several fold higher than that into the control. The OD pattern is identical in both samples but the ^{32}P profile is clearly distorted in the contaminated sample. In noncontaminated seedlings (fig 1b) the radioactivity profile matches the OD profile closely. In the contaminated seedling (fig 1a) this is clearly not so. The DNA-RNA region (tubes 50–57) is much more heavily labeled than in the


a) bacteria added
b) control
OPTICAL DENSITY, 260 nm
NaCl MOLARITY
10^{-3} x cpm
TEST TUBE NO.
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.4
0.6
0.8
1.0
1.2
0
20
40
60
80
100
120
2
4
6
8
10
12

control and the radioactivity peak corresponding to rapidly metabolized RNA clearly preceeds the OD peak. An analogous finding has recently been reported by Lonberg-Holm (13). An even more striking irregularity is seen in the s-RNA region. Between the s_1 and s_2 components [the s_2 component of the OD profile is probably not s-RNA but 5s ribosomal RNA (3, 7)] a sharp peak of radioactivity (tubes 31–39) appears. This peak corresponds to the rapidly labeled s-RNA described previously, and in our material it can be accounted for by the 4s (soluble) RNA of bacteria (18). In the contaminated sample the radioactivity profile also preceeds the OD profile in the ribosomal RNA region (tubes 66–100) as might be expected from the differences in the sedimentation constants of bacterial (16s and 23s) and plant (18s and 28s) ribosomal RNAs (2, 18).

From the present work we can not provide any clue about the so-called m-RNA (6) but further investigations are dealing with this problem.

The data presented here suggest that the reduction of $^{32}PO_4$ uptake by tris (12) seems to be mainly due to the antibacterial effect of tris, whereas sucrose, NH_4-citrate, and Mg^{2+}, of course, favor bacterial growth. Furthermore the application of Penicillin or Streptomycin even in high concentrations proved to be without effect upon the bacterial growth in this system.

In these experiments with watermelon cotyledons the deliberate introduction of bacterial contamination elicited the appearance of radioactive peaks in the s-RNA and DNA-RNA regions to which special significance has previously been attached. Clearly the possibility is still open that non-matching of OD and radioactivity profiles does occur in sterile tissues under particular conditions. Up to the present, however, we have not made such an observation. Together with the work of Lonberg-Holm (13) our data emphasize the essentiality of efficient aseptic precautions in experiments on labeling nucleic acids in plants with isotopes.

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Fig. 1. The influence of bacterial contamination (1a) upon the incorporation of $^{32}PO_4$ into nucleic acids of watermelon seedlings (1b). 50 OD_{260} units (ca 2.5 mg nucleic acids) were taken from a total of 102 OD units (1a) or 106 OD units (1b), respectively, representing the total nucleic acids extracted from 40 cotyledons at day 1 and one-half. The separation was done by the phenol procedure and the fractionation by chromatography on MAK columns. A linear gradient of NaCl ranging from 0.35 M to 1.20 M in 0.01 M tris, pH 6.9 was used. Fractions of 5 ml were collected. A total of 500 ml were eluted in 2 hours using a peristaltic pump to increase the pressure. ●—●—● OD_{260} per ml; ○...○...○... cpm per ml; ▼--▼--▼ NaCl concentration of the elution medium.

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