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SHORT COMMUNICATION

DIFFERENCES IN THE LEVEL OF PLASTID-SPECIFIC *t*RNA's IN CHLOROPLASTS AND ETIOPLASTS OF *PHASEOLUS VULGARIS*

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Abstract—Comparison of the chromatographic profiles of chloroplast and etioplast leucyl-*t*RNA's and valyl-*t*RNA's shows that the levels of plastid-specific *t*RNA species are relatively higher in the chloroplasts. This suggests that light can stimulate the synthesis of plastid-specific *t*RNA's in higher plants.

INTRODUCTION

CHLOROPLASTS of higher plants have been shown to contain *t*RNA species different from those present in the cytoplasm of the same organism, particularly *N*-formyl-methionyl-*t*RNA,[1,2] leucyl- and valyl-*t*RNA's.[3] In the case of leucyl- and valyl-*t*RNA's from bean chloroplasts, we have been able to show that the plastid-specific *t*RNA species are only aminoacylated by chloroplast enzymes and are not recognized by cytoplasmic enzymes.[3] It was of interest to study whether these plastid-specific *t*RNA's are present already in etioplasts (obtained from plants grown in the dark) or if they appear only in chloroplasts after the plants have been exposed to light.

RESULTS AND DISCUSSION

The elution profiles after co-chromatography of chloroplast and etioplast leucyl-*t*RNA's are shown on Fig. 1b, whereas Fig. 1a shows the elution profile of cytoplasmic leucyl-*t*RNA's for comparison. Six peaks of leucyl-*t*RNA's are present in both types of plastids, but their relative levels are different. If peak I (which is present in the cytoplasm as well as in both types of plastids) is taken as a reference and if the ratio of the levels *t*RNA chloroplast/*t*RNA etioplast for this peak is considered to be 1, it can be seen that the level of leucyl-*t*RNA in chloroplasts is decreased (as compared to that in the etioplasts) for peak II, but is increased in peaks III and IV (taken together) as well as in peaks V and VI.

The elution profiles after co-chromatography of chloroplast and etioplast valyl-*t*RNA's are shown in Fig. 2b, whereas Fig. 2a shows the elution profile of cytoplasmic valyl-*t*RNA's for comparison. Five peaks are present in both types of plastids, but here also their relative levels are different. Here peak II was taken as a reference, as it is present not only in the plastids but also in the cytoplasm. If the ratio of the levels *t*RNA chloroplast/*t*RNA etioplast for this peak is considered to be 1, one can see that the level of the valyl-*t*RNA in

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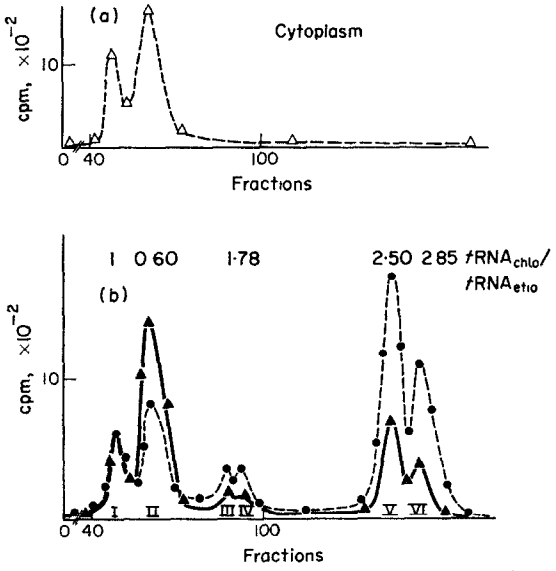

FIG. 1. REVERSE PHASE CHROMATOGRAPHY (RPC 2) OF LEUCYL-*t*RNA'S.
(a) △ cytoplasmic ^{14}C leucyl-*t*RNA's charged with cytoplasmic enzyme; (b) ● chloroplast ^{3}H leucyl-*t*RNA's charged with chloroplast enzyme; ▲ etioplast ^{14}C leucyl-*t*RNA's charged with etioplast enzyme. NaCl gradient from 0·35 to 0·7 M in NaOAc buffer 0·01 M (pH 4·7) $MgCl_2$ 0·01 M.

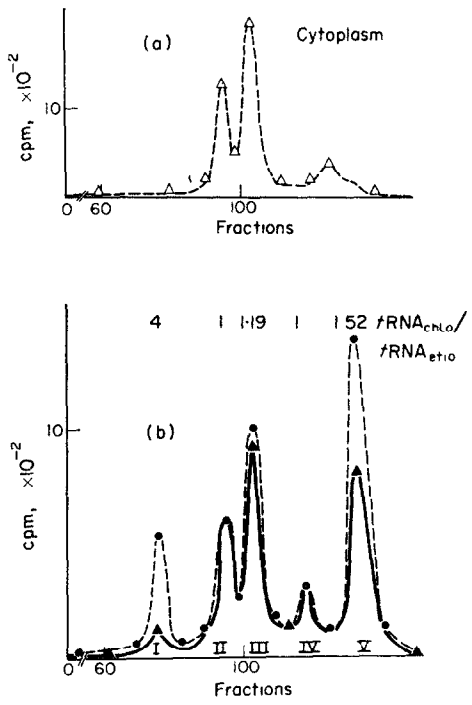

FIG. 2. REVERSE PHASE CHROMATOGRAPHY (RPC 5) OF VALYL-*t*RNA'S.
(a) △ cytoplasmic ^{14}C valyl-*t*RNA's charged with cytoplasmic enzyme; (b) ● chloroplast ^{3}H valyl-*t*RNA's charged with chloroplast enzyme; ▲ etioplast ^{14}C valyl-*t*RNA's charged with etioplast enzyme. NaCl gradient from 0·4 to 0·8 M in NaOAc buffer 0·01 M (pH 4·7) $MgCl_2$ 0·01 M.

the chloroplasts is increased (as compared to that in the etioplasts) very strongly for peak I, and to a lesser extent for peak V.

Our results show that the levels of plastid-specific leucyl-*t*RNA's III, IV, V and VI are all higher in chloroplasts as compared to etioplasts. In the case of valyl-*t*RNA's the levels of plastid-specific peaks I and V are also relatively higher in the chloroplasts. This suggests that, in a higher plant such as *Phaseolus vulgaris*, light can stimulate the synthesis of plastid-specific *t*RNA species. Our results can be compared to those of Barnett *et al.*[4,5] who have reported the existence of light-induced isoleucyl- and phenylalanyl-*t*RNA species in algal chloroplasts. Furthermore Williams and Williams[6] have observed that only certain $tRNA^{leu}$ isoacceptors are synthesized preferentially upon greening of etiolated leaves. More recently Merrick and Dure[7] have reported an increase in certain isoaccepting $tRNA^{met}$, $tRNA^{val}$ and $tRNA^{ile}$, and have suggested that the species which increase in relative concentration may be localized in the chloroplasts. Our experiments, performed with *t*RNA's obtained from

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isolated etioplasts and chloroplasts, demonstrate a preferential synthesis of plastid specific *t*RNA species upon greening.

EXPERIMENTAL

Chloroplasts as well as etioplasts have been obtained respectively from green and from etiolated leaves by a non-aqueous technique[8]; whereas the chloroplasts are found at the upper part of a layer whose density is 1·32, this layer should have a density of 1·35 in order to collect the etioplasts at its upper part. Preparations of *t*RNA's and enzymes, and aminoacylation of *t*RNA's were performed as previously described.[3] Co-chromatography of chloroplast and etioplast leucyl-*t*RNA's was performed using the reverse phase chromatographic method RPC-2,[9] but for the comparison of valyl-*t*RNA's we used the method RPC-5 which gives an improved resolution.[10]

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Key Word Index—Phaseolus vulgaris; Leguminosae; leucyl-*t*RNA's; valyl-*t*RNA's; chloroplasts; etioplasts.