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Note

Cytokinin determination in tRNA by fused-silica capillary gas chromatography and nitrogen-selective detection

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Recently, we reported results on analysis of synthetic samples of cytokinins and other modified ribonucleosides using high-resolution fused-silica capillary gas chromatography (GC)[1,2]. We have now applied these procedures, modified by the use of a nitrogen–phosphorus detector (NPD), to analysis of the natural cytokinin content of a sample of tRNA.

EXPERIMENTAL

A 100-mg amount of tRNA from wheat germ (Sigma; 18.6 A_{260} units/mg) was hydrolyzed with snake venom and alkaline phosphatase according to the procedure of Hall[3] as modified by Murai *et al.*[4]. The solid residue of ribonucleosides obtained from the reaction was extracted five times with 10-ml portions of water-saturated ethyl acetate[5] and the combined extracts were evaporated in a 2-ml reaction vial and then dried *in vacuo* over P_2O_5. Next, 375 μl ethyl acetate and 125 μl bis(trimethlsilyl)trifluoro acetamide were added and the mixture was silylated at 150°C for 30 min. Trimethylsilyl (TMS) derivatives of standard cytokinins were prepared using the same method.

Cytokinins were analyzed on a 30 m × 0.25 mm SE-54 fused-silica capillary column operated in the splitless mode as decribed[2], except that a Hewlett-Packard Model 19304B NPD at 280°C was used in place of a flame ionization detector (FID).

RESULTS AND DISCUSSION

The NPD was more sensitive to silylated ribonucleosides than the FID used in earlier work. Estimates based on GC peak areas indicated that there was an 8-fold to 10-fold greater response using NPD (0.1 nanogram range) than with FID for TMS

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derivatives of *trans*-ribosylzeatin (t-ZR) and N^6-methyladenosine, respectively. Recent work[6] with permethylated cytokinins showed that 25-pg quantites of these compounds could be detected with an NPD, although the derivatization procedure is somewhat more complex. In the analysis of biological samples for ribonucleosides, the NPD system is useful to indicate the presence of nitrogenous compounds requiring additional characterization.

Analysis of a 0.5-μl aliquot of the silylated residue from the tRNA extract (Fig. 1) shows a peak that elutes at 44 min which has the same retention time as the cytokinin *cis*-ribosylzeatin (c-ZR). This peak was well separated from all other components obtained from the reaction mixture.

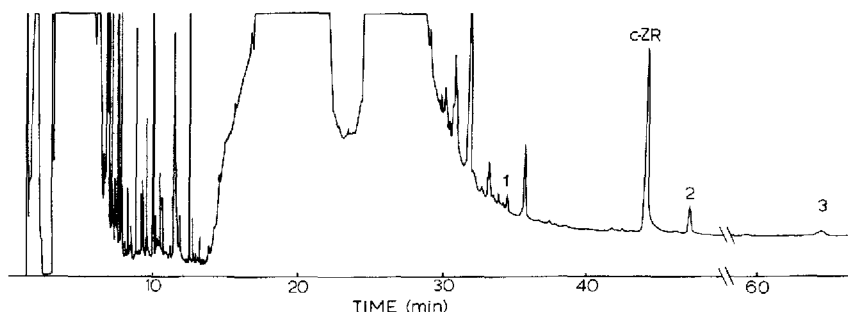

Fig. 1. Isolation of *cis*-ribosylzeatin (c-ZR) from ethyl acetate extract of enzymatically hydrolyzed tRNA. 0.5 μl of silylated sample (100 μg equivalent tRNA) separated on 30 m × 0.25 mm SE-54 fused-silica capillary column using splitless procedure and NPD. Additional peaks which co-chromatographed with authentic samples of known naturally occurring cytokinin-active ribonucleosides: 1 = N^6-(Δ^2-isopentenyl)-adenosine; 2 = *trans*-ribosylzeatin and 2-methylthioisopentenyladenosine; 3 = *cis*-2-methylthioribosylzeatin.

Chemical ionization mass spectral analysis[2] of the compound gave the same spectral pattern as that from authentic TMS_4-c-ZR with characteristic ions at *m/e* 640 $(M+H)^+$, 624 $(M-CH_3)^+$ and 550 $(M-OSi(CH_3)_3)^+$. Silylated authentic samples of all cytokinins known to occur in tRNA[1] including N^6-(Δ^2-isopentenyl)adenosine (IPA), the earliest eluting standard, separated from the major contaminants of the tRNA hydrolyzate on the SE-54 column. The major components in the chromatogram are probably adenosine and other parent and modified ribonucleosides; these compounds are the principal constituents of tRNA. Burrows *et al.*[7] have identified three additional cytokinins in wheat germ tRNA including IPA and the 2-methylthio derivatives of isopentenyladenosine (ms IPA) and *cis*-ribosylzeatin (c-msZR). Small peaks were observed in the high-resolution system (Fig. 1) which co-chromatographed with these compounds (msIPA and t-ZR co-chromatographed on the SE-54 column). c-ZR was the most abundant cytokinin in the tRNA sample; the estimated quantity of this compound recovered was 250 ng per mg tRNA. The quantities of tRNA species may differ from one commercial lot of tRNA to another so the quantity of cytokinin may vary among lots.

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