CYTOKININS: ISOLATION AND IDENTIFICATION OF 6-(3-METHYL-2-BUTENYLAMINO)-9-β-D-RIBOFURANOSYLPURINE (2iPA) FROM YEAST CYSTEINE tRNA

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SUMMARY

A cytokinin in yeast cysteine tRNA has been isolated as the riboside and has been shown to have uv and mass spectra identical with those of synthetic 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine.

The highly active cytokinin 6-(3-methyl-2-butenylamino)purine (2iP) and its riboside (2iPA)(I), synthesized in 1961 and 1963 respectively (Cavé, 1962; Cavé et al., 1962; Leonard, 1963; Leonard and Fujii, 1964; Skoog et al., 1967), have been found as constituents of the following pure tRNA's: yeast serine tRNA I and II (Zachau et al., 1966; Biemann et al., 1966) yeast tyrosine tRNA (Madison et al., 1967; Madison and Kung, 1967), and rat liver serine tRNA (Staehelin et al., 1968). Recently, cytokinin activity has been found to be associated with yeast cysteine tRNA (Armstrong et al., 1969). The isolation and identification of a cytokinin-active component of yeast cysteine tRNA as 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (2iPA) is here reported.

MATERIALS AND METHODS

Crude Saccharomyces cerevisiae (brewer's yeast) tRNA was obtained from Boehringer und Sohne, Mannheim. Fractionation of the crude tRNA on a BD-

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cellulose column has been described (Gillam et al., 1967; Armstrong et al., 1969). Fraction G from this column (see Fig. 2 of Armstrong et al., 1969) contained the cysteine acceptor activity. For the isolation of the cytokinin-active component of cysteine tRNA, a tRNA fraction highly enriched in cysteine tRNA has been prepared by rechromatography of a sample of fraction G on BD-cellulose. Details of the column fractionation procedure are given with Figure 1.

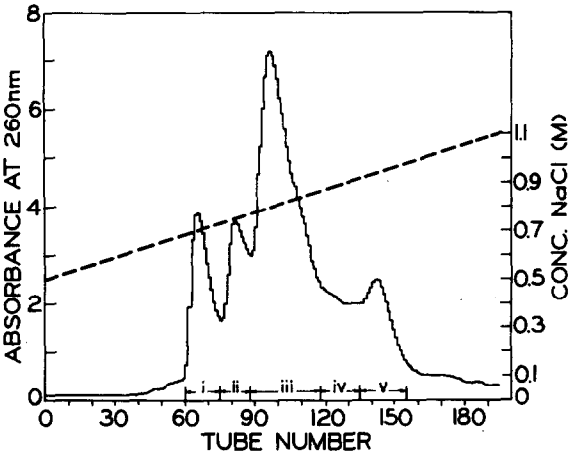


Figure 1. The elution profile following rechromatography of fraction G (see text) on a BD-cellulose column. A sample (4480 A_{260} units) of fraction G was loaded on a BD-cellulose column (1.4 x 110 cm) equilibrated with 0.05 M acetate buffer, pH 4.0, containing 0.01 M EDTA and 0.5 M NaCl. The column was eluted with the indicated salt gradient in the same buffer. The total volume of eluate was 2.6 l., and the flow rate was 80 ml/hr. The indicated fraction contained the cysteine tRNA and was used as the source of the cytokinin-active nucleoside (fraction v).

Enzymatic hydrolysis of tRNA was carried out according to Hall (1964) with lyophilized snake venom (Crotalus adamanteus, Sigma) and alkaline phosphatase (calf intestinal mucosa, Type II, Sigma).

The lyophilized tRNA hydrolysate was extracted nine times with the upper phase of ethyl acetate:water (5:1). The combined extracts were evaporated and redissolved in 35% ethanol for application to a Sephadex LH-20 column. Details of Sephadex LH-20 chromatography are given with Figure 2. All organic solvents were redistilled before use.

For determination of the cytokinin activity, a 1-ml sample was taken from each tube of the LH-20 column eluate, evaporated to dryness, acid-hydrolyzed in 0.1 N HCl at 100° for 45 min, and incorporated in the tobacco bioassay medium (Skoog et al., 1966).

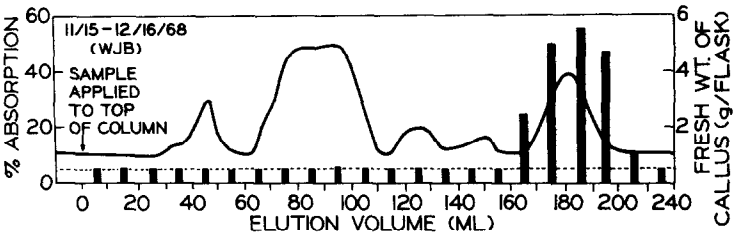


Figure 2. Chromatography of ethyl acetate soluble nucleosides from the cysteine tRNA hydrolysate. The sample, dissolved in 1 ml. of 35% ethanol, was applied to a Sephadex LH-20 column (20.5 x 2.5 cm) equilibrated with the same solvent. The column was eluted with 35% ethanol at a flow rate of 45 ml/hr. The eluate was monitored at 254 nm and fractions of 10 ml were collected. The apparent high background from the column represents a base line adjustment; the actual absorption was negligible. Cytokinin activities (dark bars) were determined as described in the text.

RESULTS AND DISCUSSION

The fractionation of a sample of fraction G (see above) by rechromatography on BD-cellulose is shown in Figure 1. Since the elution profile for this column closely resembled that of a smaller column reported by Armstrong et al. (1969), no amino acid acceptor assays were considered necessary. The fractions containing cysteine tRNA were pooled, yielding 18 mg of tRNA. This tRNA was hydrolyzed to nucleosides, and the lyophilized hydrolysate was extracted with ethyl acetate. Only traces of cytokinin activity remained in the residue.

The ethyl acetate extract was fractionated on a column of Sephadex LH-20 as shown in Figure 2. Cytokinin activity was associated with the last uv-absorbing peak eluted from the column. Fractions containing this peak were pooled, lyophilized, dissolved in 95% ethanol, and chromatographed on acid washed Whatman No. 1 paper with 10% ethanol (ascending). The uv-absorbing band (R_f 0.55-0.67) was eluted with 95% ethanol. The white solids from this eluate were used for the identification of the cytokinin.

The low resolution mass spectrum of the naturally occurring cytokinin compared favorably with a spectrum of synthetic 2iPA (Figure 3). High resolution mass spectrometry of the natural material gave m/e values of 335.159 (M^+), verifying the molecular formula as $C_{15}H_{21}N_5O_4$, and 246.137, 232.117, 203.120, 188.095, 160.061, 148.061, and 135.057, corresponding to the prominent fragment ions in the spectrum of 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine. The qualitative ultraviolet spectra of natural and

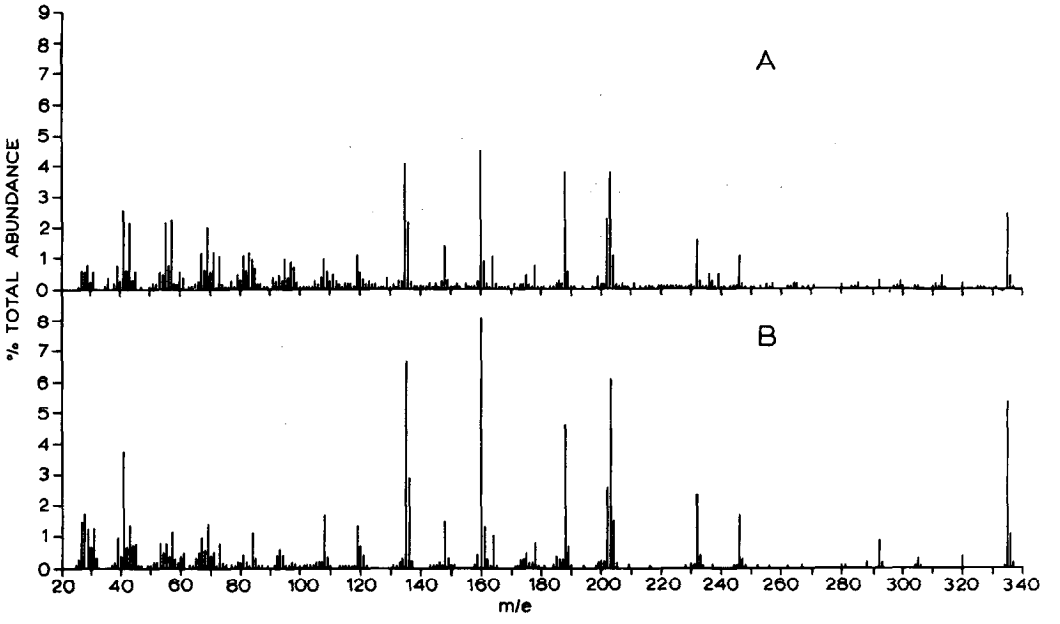
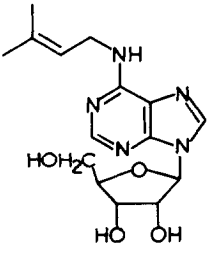


Figure 3. Mass spectral comparison at 70eV of (A) cytokinin-active nucleoside from cysteine tRNA and (B) synthetic 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine.

synthetic material were comparable in acid, neutral and basic solution, and the chromatographic values were the same for each in a number of systems.



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Tests of tRNA species from various organisms (Skoog, 1968; Skoog and Leonard, 1969; Armstrong et al., 1969 a,b) indicate that only those species which recognize U as the first codon letter possess cytokinin activity according to the tobacco bioassay. The finding of 2iPA in yeast cysteine tRNA is in agreement with this rule.

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