CYTOKININ ACTIVITY IN $tRNA^{Phe}$

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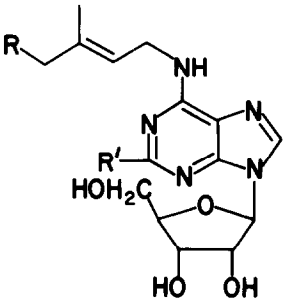
SUMMARY

It has been found that $tRNA^{Phe}$, which was thought not to have a cytokinin in the anticodon-adjacent position due to the presence of an alternate form of modification, does contain a cytokinin or cytokinin precursor.

The numerous modified nucleosides which are known to occur in transfer ribonucleic acids (tRNA's) include four compounds (1-4) which promote cell division and growth (cytokinin) activity in certain excised plant tissues.[1-6] Cytokinin testing of acid hydrolysates of amino acyl specific tRNA's has indicated that cytokinin activity is confined to those species which respond to codons beginning with uridine.[7] In Escherichia coli, for example, testing indicated the presence of cytokinin activity in tRNA's corresponding to every amino acid which had at least one codon beginning with uridine.[8] Determination of the primary sequences of a number of tRNA's has indicated that the cytokinins, where they occur, occupy the position adjacent to the 3'-end of the anticodon triplet.[1]



1, R = R′ = H
2, R = OH, R′ = H
3, R = H, R′ = SCH_3
4, R = OH, R′ = SCH_3

It has been reported, on the basis of cytokinin testing in the tobacco bioassay, that the phenylalanine and tryptophan specific tRNA's from yeast (Saccharomyces lactis) contained no cytokinin.[7] In $tRNA^{Phe}$, an unusual fluorescent nucleoside Y^{9-14} is present in the anticodon-adjacent position. It seemed, therefore, that $tRNA^{Phe}$ from yeast represented an exception to the pattern of occurrence of cytokinins in tRNA species responding to codons beginning with uridine.

We wish to report that the acid-promoted excision[11] of the fluorescent nucleosides Y from yeast and wheat germ phenylalanine tRNA's[13] resulted in compounds which were active as cytokinins.

MATERIALS AND METHODS

In each case, the tRNA's were dissolved in water (25 ml/g tRNA), dialyzed against 0.1 N ammonium formate buffer, and extracted with portions of chloroform. The solution was adjusted to pH 2.9 and incubated at 37° for four hours, according to the method of Thiebe and Zachau.[11] Neutralization of the cooled solutions, followed by extraction of the aqueous layer with chloroform, afforded the fluorescent products. The products were immediately frozen at -20° and used to record computer time-average 100 MHz nuclear magnetic resonance (nmr) spectra in $CDCl_3$ at -20°

The amount of material tested in the tobacco bioassay was estimated from the quantitative ultraviolet spectra of the products. The short wavelength maximum (240 nm) in each spectrum was assumed to have a molar absorptivity (ε) similar to that of Y; the molar absorptivity of Y was determined from

the published spectra of ApY and Y.[9] The short wavelength maximum in ApY was due largely to Y, while the maximum at 260 nm was due largely to A (the exact portion of the latter due to A was determined from a difference spectrum of ApY and Y). Therefore, ignoring contributions from hypochromicity, a rough value could be assigned for the molar absorptivity of Y, based on the molar absorptivity of A and the relative absorbance of an equimolar solution of A and Y (as ApY). Calculations of the theoretical amount of Y available for excision, based on the amount of $tRNA^{Phe}$ isolated from crude tRNA by fractionation, were in reasonable agreement with the amount actually estimated to be found.

RESULTS AND DISCUSSION

tRNA (2.0 g) derived[4,5] from wheat germ was used to record a time-averaged 100 MHz nmr spectrum in $CDCl_3$ at -20°. When the same sample was allowed to warm to 28°, a different nmr spectrum was obtained. The process was irreversible and the new spectrum indicated that none of the original material was present after warming, so that at least one new product had been formed. Paper chromatography of the new product in ethyl acetate - 1-propanol - water (4:1:2, v/v/v, upper phase) afforded fluorescent bands at R_f 0.68 and R_f 0.96. Paper chromatography of freshly extracted Y_w^+ (base moiety of Y) in the same solvent system afforded the same two bands. The material eluted from the band at R_f 0.68 showed slight cytokinin activity at high concentrations, while that eluted from the band of R_f 0.96 was at least 3% as active as 6-(3-methyl-2-butenylamino)purine.[15]

tRNA (1.0 g) derived from yeast (Saccharomyces cerevisiae, strain Y185) grown on minimal media in continuous culture with limiting glucose to force aerobic growth and shown to be free from bacterial contamination was used for the isolation of Y^+. Paper chromatography of the product in ethyl acetate 1-propanol - water (4:1:2, v/v/v) afforded fluorescent bands at R_f 0.84, 0.89 and 0.96. Cytokinin testing indicated that the compound eluted from the band at R_f 0.84 was about 0.1% as active as 6-(3-methyl-2-butenylamino)-

purine. Treatment with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide at 80° for 30 minutes did not change the level of activity. The compound eluted from the band at R_f 0.89 was about half as active as the material from the band at R_f 0.84. Acid treatment had no effect on activity, but base treatment reduced activity slightly. The compound eluted from the band at R_f 0.96 was inactive as a cytokinin. Acid treatment had no effect on activity, but base treatment produced a compound with about one third the activity of the material from the band at R_f 0.84.

Y^+ was also isolated from $tRNA^{Phe}$ derived from commercial baker's yeast. The product which was initially isolated was used to record a 100 MHz nmr spectrum at -20° in $CDCl_3$. The spectrum, which was indicative of nuclear side-chain substitution, changed when the solution was warmed. Paper chromatography of the original isolate afforded fluorescent bands at R_f 0.80 and R_f 0.96.

The finding of cytokinin activity has definite structural implications for Y; these are indicated in the structure-activity literature on cytokinins.[6,16–19] High cytokinin activity in the purine series has been limited to N^6-substituted adenines. C_5 substituents and planarity of the substituent side chain have afforded the highest degree of cytokinin activity. Substitution at the 2- or 8-position has relatively little effect, but alteration of the adenine moiety, or substitution at any of the other positions, causes more substantial reduction in cytokinin activity. A C_{10} side chain, or two C_5 side chains, attached through N^6, would lower cytokinin activity relative to that of 6-(3-methyl-2-butenylamino)purine.[6] One should note that it might not be possible to differentiate between isomeric compounds, one with two C_5 side chains and one with a single C_{10} side chain, solely on the basis of mass spectrometry.[20]

On the basis of these experiments, it seems reasonable to assert that (1) the fluorescent materials derived by acid excision from $tRNA^{Phe}$ (from wheat germ and two types of yeast) are all different and appear to be un-

stable, (2) the fluorescent materials, and hence the anticodon-adjacent nucleosides in the tRNA's, are cytokinins or cytokinin precursors, and (3) the fact that Y is a cytokinin or cytokinin precursor imposes a number of specific constraints on the possible structures which may correspond to Y.

It is gratifying to note that $tRNA^{Phe}$, which was thought not to have a cytokinin because of the presence of an alternate form of modification in the anticodon-adjacent position, does indeed contain a cytokinin or cytokinin precursor.

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