# UNIQUE PRESENCE OF 2-METHYLTHIO-RIBOSYLZEATIN IN THE TRANSFER RIBONUCLEIC ACID OF THE BACTERIUM PSEUDOMONAS AERUGINOSA

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SUMMARY: Analysis of $^{35}S$ labled nucleosides prepared from tRNA of Pseudomonas aeruginosa by phosphocellulose column chromatography, thin layer chromatography and Sephadex LH-20 column chromatography revealed the presence of 2-methylthio-ribosylzeatin in it.

tRNA hydrolysates from a variety of organisms have been found to contain cytokinin-active ribonucleosides (1-5). In the tRNA of Escherichia coli 2-methylthio $N^6$-isopentenyladenosine (ms-2iPA) predominates and with isopentenyladenosine (2iPA), accounts for the bulk of the observed cytokinin activity (6-8). Yeast (9) and Staphylococcus epidermis (10) tRNAs appear to contain mainly 2iPA. However in tRNAs of higher plants such as spinach and peas, ribosyl-cis-zeatin are reported to be present (11,12). Ribosyl-cis-zeatin is also the predominant cytokinin in tRNA from wheat germ (2,13,14) and tobacco callus tissue grown in a medium containing 6-benzylaminopurine (15) but ms-ribosylzeatin and 2iPA have also been found in

Abbreviations:- 2iPA : 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosyl purine; ms-2iPA: 6-(3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine; ribosyl-cis-zeatin: 6-(4-hydroxy-3-methyl-cis-2-butenylamino)-9-β-D-ribofuranosyl-purine; ribosyl-trans-zeatin: 6-(4-hydroxy-3-methyl-trans-2-butenylamino)-9-β-D-ribofuranosylpurine; ms-ribosylzeatin: 6-(4-hydroxy-3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine. The term used here does not distinguish between cis and trans isomers of this compound. $s^4U$: 4-thiouridine; $s^2U^*$: 5-methylaminomethyl-2-thiouridine; $s^2C$: 2-thiocytidine; TLC - thin layer chromatography.

these preparations. Young green pea shoots are also reported to contain ribosyl-zeatins, both cis and trans isomers, ms-ribosylzeatin, 2iPA and ms-2iPA (16). Therefore evidence available in literature shows that ribosylzeatins are present in the tRNAs of higher plants while ms-2iPA or 2iPA are the constituents of bacterial tRNA, although recently a cis-zeatin was isolated from culture medium of Corynebacterium fascians, a plant pathogen (17). In this communication we present evidence for the presence of ms-ribosylzeatin so far considered to be of plant origin, in the tRNA of Pseudomonas aeruginosa. This is the first report of the presence of a zeatin-riboside in the tRNA of a bacterium.

METHODS AND MATERIALS: Growth of Pseudomonas aeruginosa and isolation of $^{35}S$ labeled tRNA was as described previously (18). Details for the preparation of $^{35}S$ nucleosides and column chromatography on phosphocellulose have already been reported (19). Enzymatic hydrolysis of tRNA, preparation of ethyl acetate-soluble ribonucleosides and Sephadex LH-20 column chromatography were performed according to the procedure followed by Burrows et al. (15). Paper chromatography was carried out on a Whatman No.1 filter paper, in the descending manner. Thin layer chromatography was carried out on glass plates coated with cellulose (0.5 mm thickness, MN 300 microcrystalline cellulose, Sigma). TLC on silica gel was carried out on Eastman chromagram sheets with fluorescent indicator. Authentic sample of ms-ribosylzeatin (trans-isomer) was chemically synthesised in Prof.N.J.Leonard's laboratory, University of Illinois, Urbana and was supplied as a gift by Prof.F.Skoog, University of Wisconsin. ms-2iPA was a gift from Dr. S.M.Hecht, M.I.T., Massachusetts.

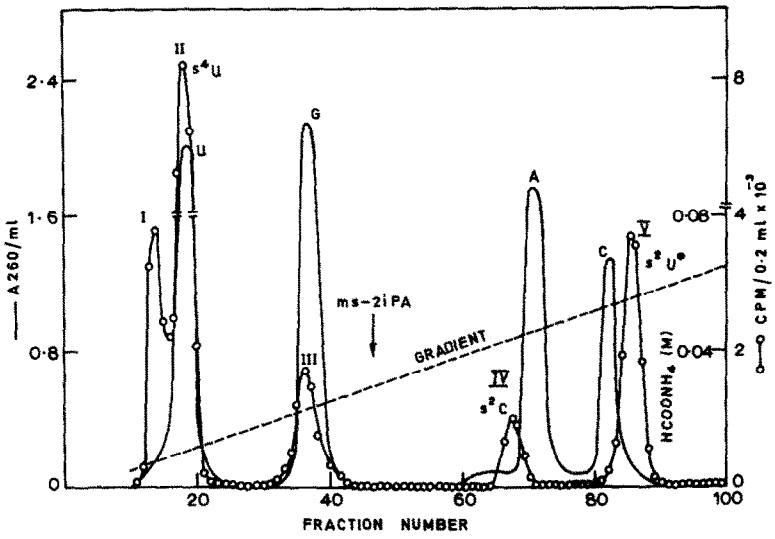


Figure 1: Phosphocellulose column chromatography of $^{35}S$ labeled nucleosides of P.aeruginosa tRNA. 10 $A_{260}$ units of $^{35}S$ labeled tRNA ($9.0 \times 10^5$ CPM) were mixed with 40 $A_{260}$ units of unlabeled P.aeruginosa tRNA, converted into nucleosides as described under Methods, and chromatographed on a phosphocellulose column (1 x 55 cm), with a linear ammonium formate gradient from 0.005 M to 0.09 M, pH 3.9 each 140 ml. Flow rate was 8 ml/hr. 2 ml fractions were collected and 0.2 ml each of the fractions was applied to filter paper discs and counted. Recovery 100%.

RESULTS: Earlier we have reported the presence of thionucleotides in P.aeruginosa tRNA (18) and recently described a simple column chromatographic procedure for quantitative separation of $^{35}S$ nucleosides on phosphocellulose (19). This procedure is highly reproducible and a comparison of $^{35}S$ nucleoside peaks observed with E.coli and P.aeruginosa tRNAs showed one major difference. ms-2iPA present in E.coli tRNA was absent in P.aeruginosa tRNA and in its place a radioactivity peak eluted slightly earlier (19). Fig.1 shows the separation of $^{35}S$ nucleosides of P.aeruginosa tRNA on a phosphocellulose column. Peaks I, II, IV and V were identified as non-nucleoside sulfur, 4-thiouridine, 2-thiocytidine and 5-methylaminomethyl-2-thiouridine respectively (19). The position for the

elution of ms-2iPA is shown by the arrow mark. As can be seen, radioactivity due to ms-2iPA was absent and in its place a new radioactivity peak appeared eluting slightly earlier to ms-2iPA. We suspected this to be a ms-2iPA derivative with hydroxylated isopentenyl side chain. This peak was pooled, evaporated to dryness, dissolved in 10% ethanol, mixed with authentic sample of ms-ribosylzeatin and subjected to paper and thin layer chromatography employing different solvent systems. As can be seen in Table I in solvent systems A, B, C and D radioactivity moved along with authentic sample. In

Table 1

$R_f$ values of radioacive peak III of phosphocellulose column (Fig. 1) and authentic sample, ms-ribosylzeatin and ms-2iPA on paper and TLC.

| Solvent system | Radioactivity | Authentic sample ms-ribosylzeatin | ms-2iPA |
|---|---|---|---|
| A. Whatman No.1 paper | 0.83 | 0.83 | 0.9 |
| A. TLC (cellulose) | 0.81 | 0.81 | - |
| B. " | 0.89 | 0.89 | - |
| C " | 0.89 | 0.89 | - |
| D " | 0.91 | 0.91 | - |
| E TLC (silica gel) | 0.34 | 0.30 | - |

A - isopropanol - Conc. $NH_3$ - water, (7:1:2 v/v/v)., B - ethanol- 1M ammonium acetate, pH 7.5 (7:3 v/v)., C - n -butanol-water (86:14, v/v)., D - ethylacetate-n-propanol-water (4:1:2, v/v/v, upper layer) and E - chloroform-methanol (9:1, v/v) TLC plates and paper were scraped or cut and counted in Beckman LS 100 counter.

paper chromatography with solvent A, ms-2iPA moved faster than ms-ribosylzeatin with a $R_f$ value of 0.9. Sephadex LH-20 column chromatography has been used by Vreman et al. (16) for the separation of different cytokinins present in the tRNA of young green pea shoots and achieved a good aeparation. We prepared ethylacetate-soluble $^{35}S$-ribonucleosides from P.aeruginosa tRNA, mixed with authentic sample of ms-ribosylzeatin and subjected the mixture to Sephadex LH-20 column chromatography. This is shown in Fig.2. As expected the authentic sample, ms-ribosylzeatin (identified by its characteristic spectrum) separated from bulk nucleosides and eluted separately. A significant proportion of $^{35}S$ radioactivity eluted along with this peak

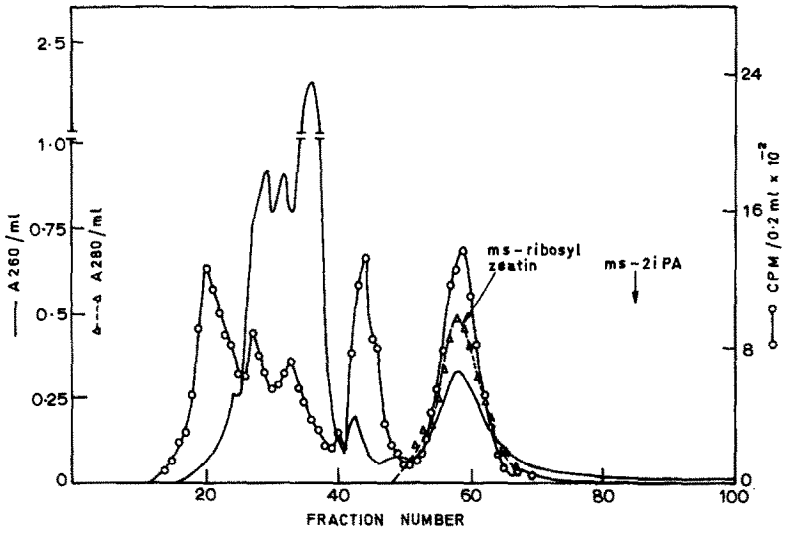


Figure 2: Sephadex LH-20 column chromatography of ethyl acetate-soluble $^{35}S$ labeled nucleosides from tRNA of P.aeruginosa. Nine $A_{260}$ units of $^{35}S$ labeled tRNA ($8.1 \times 10^5$ CPM) were mixed with 40 $A_{260}$ units of unlabeled tRNA, converted to nucleosides enzymatically as described under Methods, extracted with ethyl acetate, mixed with 4 $A_{260}$ units of authentic sample of ms-ribozylzeatin, evaporated to dryness, dissolved in 0.5 ml of 33.3% ethanol, and applied to a Sephadex LH-20 column (0.5 x 45 cms). Elution was with 33.3% ethanol, flow rate was 2.4 ml/hr. 0.2 ml fractions were collected, the fractions were diluted to 1.2 ml and 0.2 ml of this fraction was taken for counting. Recovery 97%.

giving an additional evidence for the presence of ms-ribosylzeatin in the tRNA of P.aeruginosa.

Ribosylzeatin is present in cis and trans isomeric forms. Studies on tRNAs of higher plants so far reported suggest the presence of cis isomers (16) except in Pisum tRNA where both isomers are shown to be present. In order to know the type of isomer present in P.aeruginosa tRNA, a known amount of radioactivity from peak III of phosphocellulose column (Fig.1), along with the authentic sample was subjected to thin layer chromatography on silica gel with a mixture of chloroform and methanol (9:1, v/v). This system is known to separate cis and trans isomers of ribosylzeatin (14). In this system the radioactive spot moved slightly faster than the authentic sample (Table 1). The authentic sample used here was trans isomer. Since the radioactivity moved with higher $R_f$ value (0.34) than the trans isomer (0.3) it is likely that the tRNA contains cis isomer. However, we cannot be certain about this as we could not check the $R_f$ value of cis isomer.

DISCUSSION: The results presented above show that P.aeruginosa tRNA contains ms-ribosylzeatin. The tRNA isolated from cells grown in logarithmic and late logarithmic stages gave identical phosphocellulose column chromatographic profiles indicating that growth conditions do not alter the thionucleotide pattern. ms-2iPA is a normal iPA derivative found in the tRNA of a wide variety of bacteria such as E.coli (6), S.epidermis (10), B.subtilis and S.typhimurium (20). It is therefore surprising that this bacteria should have zeatin riboside in its tRNA. Pseudomonas is a versatile genus, known both for its biosynthetic and biodegradative abilities (21).

The genus consists of saprophytes and animal as well as plant pathogens, although the strain P.aeruginosa is not a plant pathogen. It is therefore interesting to investigate whether plant pathogenic bacteria have zeatin ribosides in their tRNAs. Work in this direction is in progress.

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