THE EFFECT UPON AMINOACYLATION OF BISULPHITE ADDITION TO 2-METHYLTHIO-N^6-ISOPENTENYL ADENOSINE OF *ESCHERICHIA COLI* PHENYLALANINE tRNA

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1. Introduction

One of the most extensively used methods to investigate the structural regions or residues of tRNA which are involved in specific aminoacylation is that of chemical modification. By this method, modification of tRNA bases by specific reagents may often be correlated with loss of amino acid accepting activity (for review see [1]). In many cases the chemical modification leads to a change in kinetic parameters rather than total loss of activity, so making interpretation of results difficult. Here we report that specific chemical modification of the hypermodified residue ms^2i^6A in *Escherichia coli* $tRNA^{Phe}$ caused no change in the kinetic parameters for aminoacylation. This permitted the unambiguous interpretation that modification of the side chain of ms^2i^6A in *E. coli* $tRNA^{Phe}$ does not affect $tRNA^{Phe}$-phenylalanyl tRNA synthetase recognition nor charging of the $tRNA^{Phe}$.

2. Materials and methods

Purification of $tRNA^{Phe}$ from mixed tRNAs of *E. coli* K12CA265 (Microbiological Research Establishment, Porton, Salisbury, England) was performed as previously described [2].

Phenylalanine tRNA (1500 pmol per A_{260} units, 5 A_{260} units ml^{-1}) in 10 mM $MgCl_2$; 1.0 M sodium bisulphite, pH 7 was incubated for 24 h at 37°C and then treated as previously described [2]. The reaction mixture (0.75 ml) for the study of the kinetics of aminoacylation contained 100 mM Tris–HCl pH 7.5; 10 mM $MgCl_2$; 10 mM KCl; 10 mM NH_4Cl; 4 mM reduced glutathione; 2 mM ATP; 6.7 μM [2,3-^{3}H]-phenylalanine (5 Ci $mmol^{-1}$); 0.4 μg of *E. coli* phenylalanyl tRNA synthetase (prepared by the method of Stulberg [3]) and different amounts (0–0.4 nmol) of treated and untreated *E. coli* $tRNA^{Phe}$. The reaction was started after 2 min preincubation at 37°C, by addition of the enzyme (0.02 ml). Samples (0.06 ml) were withdrawn at 20-s intervals, pipetted onto 2.5 cm Whatman 3 MM filter discs, which were added to 10% (w/v) trichloracetic acid, washed three times in 5% trichloracetic (w/v) acid, once in ethanol and then dried. The [^{3}H]phenylalanine was solubilized in 0.5 ml 10% (v/v) hyamine hydroxide (60°C; 20 min) and counted in 0.5% (w/v) PPO/0.03% (w/v) POPOP/toluene scintillation fluid.

3. Results and discussion

The initial rate of aminoacylation, v, was determined from the slope of the linear plots of extent of aminoacylation vs. time, for different concentrations, s, of each $tRNA^{Phe}$ sample. From the Lineweaver-Burke [4] plots of $1/v$ vs. $1/s$, the reciprocal of the $tRNA^{Phe}$ concentration, the Michaelis constants, K_m, for the untreated and bisulphite (pH 7) reacted $tRNA^{Phe}$ were determined (fig.1). Values obtained were 2.5×10^{-7} M in both cases, i.e., close to the value of 2.6×10^{-7} M obtained by Stulberg [3]. The corresponding values of maximal initial velocity, V, for the above reaction conditions were 14.3 pmol Phe accepted min^{-1} (ml reaction mixture)$^{-1}$ for both forms of $tRNA^{Phe}$.

We have previously shown that, under the conditions used for modification, no deamination of accessible cytidine residues occurs [2]. At this pH, bisulphite reacts with accessible uridine residues to form 5,6-dihydrouridine-6-sulphonate residues but these are readily reconverted to uridines during the subsequent treatment with Tris–HCl (pH 9.0). However, 'fingerprints' of ribonuclease T_1 – or pancreatic ribonuclease digests of bisulphite (pH 7.0) treated *E. coli* $tRNA^{Phe}$ differed from those of untreated $tRNA^{Phe}$.

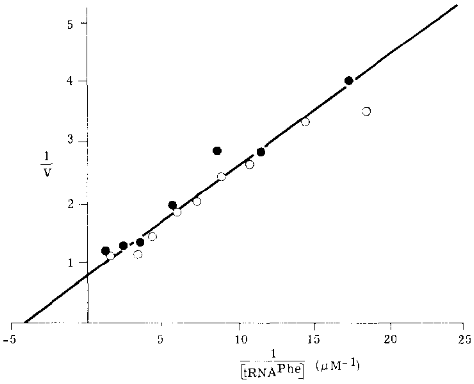

Fig.1. The effect of variation of concentration of *E. coli* $tRNA^{Phe}$ (○) or bisulphite (pH 7)-reacted $tRNA^{Phe}$ (●) on the rate of formation of phenylalanyl $tRNA^{Phe}$. Initial velocities are expressed as pmol Phe accepted min^{-1} (ml of reaction mixture)$^{-1}$. Further details are described in Materials and Methods.

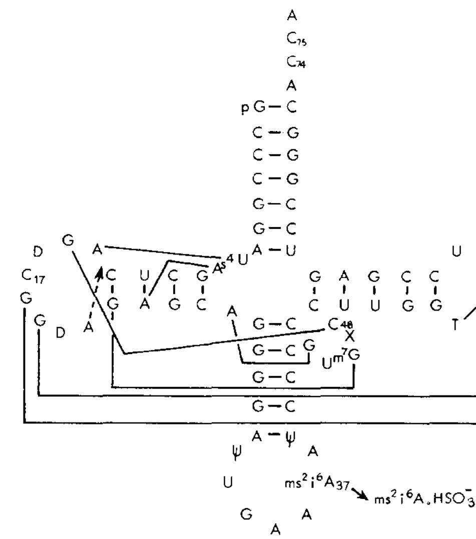

Fig.2. The structure of *E. coli* $tRNA^{Phe}$ showing the site of reaction of sodium bisulphite at pH 7.0.

The ribonuclease T_1 product, A–A–ms^2i^6A–A–Ψ–C–C–C–C–Gp decreased drastically and its loss was quantitatively compensated for by a new spot A–A–N–A–Ψ–C–C–C–C–Gp. Similar results were obtained for the pancreatic ribonuclease product G–A–A–ms^2i^6A–A–Ψp and its derivative G–A–A–N–A–A–Ψp. Subsequent analysis of ^{32}P-labelled $tRNA^{Phe}$ reacted with 1 M sodium [^{35}S]bisulphite, pH 7.0, showed that the new alkali-stable product, N, contained one bisulphite molecule per residue. This suggested that the new product is analogous to that found when N^6-isopentenyl adenosine is modified by anti-Markownikoff addition of a sulphite radical to the unsaturated side-chain [5] (fig.2).

The residue ms^2i^6A is found adjacent to the 3′-end of the anticodon of tRNA molecules which recognise a codon with a 5′-terminal uridine [6]. More recently it has been shown to play a role in the regulation of aromatic aminoacid transport by tRNA [7]. Work by Faulkner and Uziel [8] has shown that iodination of *E. coli* $tRNA^{Phe}$ caused inactivation of the tRNA in polyphenylalanine synthesis but only a small loss of amino acid acceptance activity. However, the precise nature of the products of iodination of s^4U and ms^2i^6A in the $tRNA^{Phe}$ was uncertain and no kinetic studies of aminoacylation were performed. Our observation provides less equivocal evidence that modification of ms^2i^6A does not affect the kinetics of aminoacylation of $tRNA^{Phe}$ and suggests that this residue does not play an important role in recognition, binding and aminoacylation of the tRNA by phenylalanyl-tRNA-synthetase. In this respect, ms^2i^6A in *E. coli* $tRNA^{Phe}$ appears to differ from the corresponding hypermodified nucleoside, Y, in yeast $tRNA^{Phe}$ since Krauss et al. [9] have demonstrated a dramatic decrease in the binding constant of this tRNA with its cognate homologous ligase when the Y base was excised.

Acknowledgement

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References

[1] Goddard, J. P. (1977) in: Progress in Biophysics and Molecular Biology (Butler, J. A. V. and Noble, D. eds) vol. 32, pp. 233–308, Pergamon, Oxford.

[2] Lowdon, M. and Goddard, J. P. (1976) Nucleic Acids Res. 3, 3383–3396.

[3] Stulberg, M. P. (1967) J. Biol. Chem. 242, 1060–1064.

[4] Lineweaver, H. and Burk, D. (1934) J. Amer. Chem. Soc. 56, 658–666.

[5] Hayatsu, H., Wataya, Y. and Furichi (1972) Chemosphere 2, 75–78.

[6] Nishimura, S. (1972) in: Progress in Nucleic Acid Research and Molecular Biology (Davidson, J. N. and Cohn, W. D. eds) vol. 12, pp. 49–85, Academic Press, London.

[7] Buck, M. and Griffiths, E. (1981) Nucleic Acids Res. 9, 401–414.

[8] Faulkner, R. D. and Uziel, M. (1971) Biochim. Biophys. Acta 238, 464–474.

[9] Krauss, G., Peters, F. and Maass, G. (1976) Nucleic Acids Res. 3, 631–639.