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AMINOACYL-tRNA SYNTHETASES FROM BAKER'S YEAST: REACTING SITE OF ENZYMATIC AMINOACYLATION IS NOT UNIFORM FOR ALL tRNAs

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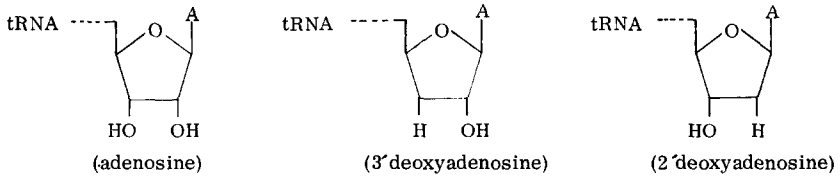
1. Introduction

In previous work we showed that $tRNA^{Phe}$ from baker's yeast was phenylalanylated exclusively at the 2'hydroxyl group of the terminal ribose by phenylalanyl-tRNA synthetase [1,2]. This was based on the observation that after incorporation of 3'deoxyadenosine and 2'deoxyadenosine, respectively, into the terminus of $tRNA^{Phe}$ only $tRNA^{Phe}$-C-C-3'dA could be aminoacylated whereas $tRNA^{Phe}$-C-C-2'dA was a competitive inhibitor of the enzyme. The same result was later obtained, by an independent method, for $tRNA^{Phe}$ from *Escherichia coli* and from rat liver [3]. These results seemed to be reasonable also from the chemical point of view as suggested by Zamecnik [4] since the 2'hydroxyl group is generally more reactive towards acylation. We then tested $tRNA^{Ile}$, $tRNA^{Ser}$*, $tRNA^{Tyr}$, and $tRNA^{Val}$ from yeast in order to check the general validity of the enzymatic aminoacylation of the more reactive 2'hydroxyl group. We found, however, three classes of tRNAs: those aminoacylated at the 2'hydroxyl group, those aminoacylated at the 3'hydroxyl group and those aminoacylatable at both hydroxyl groups.

2. Materials and methods

The baker's yeast enzymes isoleucyl- (EC 6.1.1.5), seryl- (EC 6.1.1.11) and valyl-tRNA synthetases (EC 6.1.1.9) were purified by affinity elution [5], tyrosyl-tRNA synthetase (EC 6.1.1.1) by an analogous procedure and tRNA-nucleotidyl transferase (EC 2.7.7.21) as published [6]. $tRNA^{Ser}$-C-C was isolated from unfractioned yeast tRNA [7]; $tRNA^{Ile}$-C-C and $tRNA^{Val}$-C-C as well as $tRNA^{Tyr}$-C-C-A were prepared using slight modifications of our procedure [7]. The terminal AMP from $tRNA^{Tyr}$-C-C-A was removed by snake venom phosphodiesterase (EC 3.1.4.1, Boehringer, Mannheim, Germany) [8]. [^{14}C]amino acids of stanstar grade (Schwarz Bioresearch, Orangeburg, USA) were used. All other materials were of highest commercially available purity.

Incorporation of 3'dAMP and 2'dAMP into the terminus of the respective $tRNA^{Xxx}$-C-C [1] was carried out by incubating a mixture of 100 mM Tris·HCl buffer pH 9.0, 100 mM KCl, 10 mM $MgSO_4$, 3 mM deoxyadenosine triphosphate, 0.05 mM $tRNA^{Xxx}$-C-C, 0.66 mg/ml bovine serum albumin and 400 units/ml tRNA-nucleotidyl transferase (spec. act. of 5 000 units/mg) at 30°C for 2 hr. In the case of incorporation of 3'dAMP into $tRNA^{Val}$-C-C incubation was continued for 16 hr with double amount of



* *Abbreviations:* $tRNA^{Ser}$, $tRNA^{Ser}$-C-C-A, native serine transfer RNA; $tRNA^{Ser}$-C-C, $tRNA^{Ser}$ lacking the terminal AMP; $tRNA^{Ser}$-C-C-3'dA and $tRNA^{Ser}$-C-C-2'dA, $tRNA^{Ser}$ with terminal 3'deoxyadenosine and 2'deoxyadenosine, respectively, instead of adenosine.

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enzyme. Sodium periodate oxidation was performed as described [9].

Aminoacylation was performed according to [10]. The assay mixture contained 150 mM Tris·HCl buffer pH 7.6, 50 mM KCl, 10 mM $MgSO_4$, 0.04 mM [^{14}C] amino acid and 0.003 mM $tRNA^{Xxx}$-C-C-N. To start the reaction an appropriate amount of corresponding aminoacyl-tRNA synthetase was added. For study of the Michaelis-Menten kinetics of aminoacylation of $tRNA^{Tyr}$ 150 mM KCl instead of 50 mM KCl was used.

3. Results and discussion

Deoxynucleotides could be incorporated into the terminal position of $tRNA^{Ile}$, $tRNA^{Ser}$, $tRNA^{Tyr}$, and $tRNA^{Val}$ as with $tRNA^{Phe}$ [1,2] although slight modifications with respect to enzyme concentration and incubation time had to be introduced for each individual tRNA. Thus 3′dATP was a rather weak substrate for incorporation into $tRNA^{Val}$ and only prolonged incubation led to a quantitatively modified $tRNA^{Val}$.

The aminoacylation data of the various tRNA derivatives are given in table 1. The $tRNA^{Xxx}$-C-C species are characterized by aminoacylation in the absence and presence of tRNA-nucleotidyl transferase and ATP. The latter values represent the maximal aminoacylation of each individual tRNA. It can be calculated that $tRNA^{Ser}$, $tRNA^{Tyr}$, and $tRNA^{Val}$ are more than 90% pure whereas $tRNA^{Ile}$ is about 80% pure (1.65 nmol/A_{260} unit corresponds to 100% aminoacylation, based on phosphate estimation). The aminoacylation in absence of tRNA-nucleotidyl transferase and ATP gives the amount of tRNAs with residual adenosine; the content of $tRNA^{Xxx}$-C-C-A in $tRNA^{Xxx}$-C-C is $< 5\%$ except for $tRNA^{Tyr}$ (14%).

After incorporation of 2′deoxyadenosine the aminoacylation values for $tRNA^{Ser}$-C-C-2′dA and $tRNA^{Tyr}$-C-C-2′dA are restored, whereas $tRNA^{Ile}$-C-C-2′dA and $tRNA^{Val}$-C-C-2′dA exhibit blank values. Since identical blank values are also observed in the presence of tRNA-nucleotidyl transferase and ATP they cannot be due to the lack of incorporation of 2′deoxyadenosine into the terminal position of $tRNA^{Ile}$ and $tRNA^{Val}$. The aminoacylation of $tRNA^{Ser}$-C-C-2′dA and $tRNA^{Tyr}$-C-C-2′dA is not sensitive to periodate oxidation, indicating that no ribose with cis-glycol group occupies the terminus of these tRNAs. These results clearly show that $tRNA^{Ser}$-C-C-2′dA and $tRNA^{Tyr}$-C-C-2′dA are aminocylated at the 3′hydroxyl group.

In contrast incorporation of 3′deoxyadenosine in the tRNAs leads to restoration of aminoacylation in the case of $tRNA^{Ile}$-C-C-3′dA, $tRNA^{Val}$-C-C-3′dA and $tRNA^{Tyr}$-C-C-3′dA. $tRNA^{Ser}$-C-C-3′dA cannot be aminoacylated. Again the activity of $tRNA^{Ile}$-C-C-3′dA, $tRNA^{Val}$-C-C-3′dA and $tRNA^{Tyr}$-C-C-3′dA is resistant to periodate treatment and the activity of $tRNA^{Ser}$ cannot be restored by tRNA-nucleotidyl transferase and ATP. Therefore $tRNA^{Ser}$ is aminoacylated exclusively at the 3′hydroxyl group of the terminal adenosine, whereas $tRNA^{Val}$ and $tRNA^{Ile}$ are exclusively aminoacylated at the 2′hydroxyl group. $tRNA^{Tyr}$, however, can accept its aminoacid on both the 3′ as well as on the 2′hydroxyl group.

Table 1
Specific activity (nmol/A_{260} units) in aminoacylation of modified tRNAs before and after periodate oxidation as well as with and without subsequent restoration of the -C-C-A end by tRNA-nucleotidyl transferase and ATP (NTase)

tRNA species	$tRNA^{Xxx}$-C-C		$tRNA^{Xxx}$-C-C-2′dA				$tRNA^{Xxx}$-C-C-3′dA			
			Before oxidation		After oxidation		Before oxidation		After oxidation	
	− NTase	+ NTase	− NTase	+ NTase	− NTase	+ NTase	− NTase	+ NTase	− NTase	+ NTase
$tRNA^{Ile}$	0.01	1.20	0.03	0.07	0.01	0.01	0.70	0.88	0.62	0.65
$tRNA^{Ser}$	0.07	1.52	1.38	1.49	1.43	1.43	0.06	0.18	0.01	0.01
$tRNA^{Tyr}$	0.23	1.63	1.46	1.50	1.22	1.26	1.42	1.42	1.14	1.14
$tRNA^{Val}$	0.02	1.43	0.04	0.10	0.01	0.01	1.00	1.07	0.92	0.95

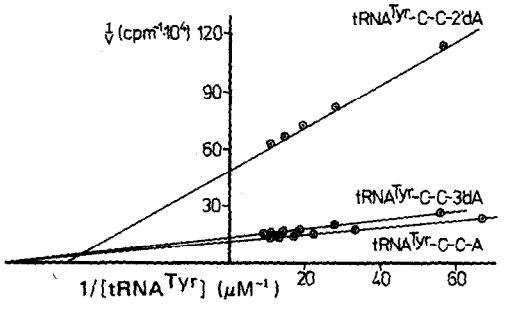

Fig.1. Aminoacylation of $tRNA^{Tyr}$-C-C-A, $tRNA^{Tyr}$-C-C-3'dA and $tRNA^{Tyr}$-C-C-2'dA. Data have been taken from the linear part of aminoacylation. With $tRNA^{Tyr}$-C-C-2'dA three times the amount of enzyme has been used. 1000 cpm are equivalent to 0.017 nmol [^{14}C] tyrosine.

Both $tRNA^{Tyr}$ derivatives were further characterized by Michaelis-Menten kinetics (fig.1). $tRNA^{Tyr}$-C-C-3'dA and $tRNA^{Tyr}$-C-C-2'dA exhibit almost the same K_M as $tRNA^{Tyr}$-C-C-A. V_{max} is about 15 times slower for $tRNA^{Tyr}$-C-C-2'dA as compared to $tRNA^{Tyr}$-C-C-A and $tRNA^{Tyr}$-C-C-3'dA. From this it can be concluded that native $tRNA^{Tyr}$-C-C-A in which both hydroxyl groups are present is aminoacylated at the 2'hydroxyl group preferentially.

There are apparently three different classes of aminoacyl-tRNA synthetases: one attaching the aminoacid to the 2'hydroxyl group of the terminal ribose, one attaching it to the 3'hydroxyl group and one using both the 2' and 3'hydroxyl group. There may be a fourth class where the presence of both the 2' and the 3'hydroxyl group at the terminus of tRNA is a prerequisite for enzymatic aminoacylation, irrespective of which hydroxyl group is aminoacylated.

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