ISOLEUCYL-tRNA SYNTHETASE FROM BAKER'S YEAST: THE 3'-HYDROXYL GROUP OF THE 3'-TERMINAL RIBOSE IS ESSENTIAL FOR PREVENTING MISACYLATION OF $tRNA^{Ile}$-C-C-A WITH MISACTIVATED VALINE

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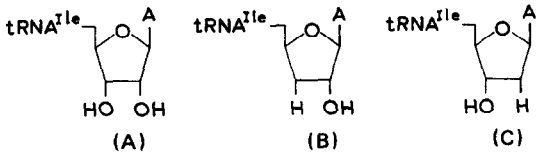
1. Introduction

It is a well established fact that several aminoacyl-tRNA synthetases are able to form an (aminoacyl-AMP·enzyme) complex with non-substrate aminoacids structurally structurally related to the substrate amino-acid (for references see [1]). One of the best document-ed cases is isoleucyl-tRNA synthetase of *Escherichia coli*, which is able to activate valine yielding Val-AMP· isoleucyl-tRNA synthetase [2]. This complex is immediately hydrolysed by the addition of $tRNA^{Ile}$-C-C-A [3,4], indicating that a correction mechanism prevents misacylation of $tRNA^{Ile}$-C-C-A. A prerequisite of this correction mechanism is the presence of an intact 3'-adenosine on $tRNA^{Ile}$, since any $tRNA^{Ile}$ modified at the 3'end – including Ile-$tRNA^{Ile}$ – lacks the ability to hydrolyse the Val-AMP·isoleucyl-tRNA synthetase complex [4]. For isoleucyl-tRNA synthe-tase from *Bacillus stearothermophilus* it is reported that at temperatures >70°C the enzyme is able to transfer misactivated valine to $tRNA^{Ile}$-C-C-A [5].

Isoleucyl-tRNA synthetase from baker's yeast shows almost the same behaviour as the enzyme from *E. coli* with respect to misactivation of valine (unpublished results from this laboratory). In an earlier report [6] we showed that the accepting hydroxyl group for the isoleucyl residue is the 2'- and not the 3'-hydroxyl group of the 3'-terminal adenosine of tRNA. This could be tested by substitut-ing the 3'-adenosine of $tRNA^{Ile}$-C-C-A (A) with 3'-deoxyadenosine (B) and 2'-deoxyadenosine (C), respectively [6].

After this modification only $tRNA^{Ile}$-C-C-3'dA (B) was isoleucylated by isoleucyl-tRNA synthetase but not $tRNA^{Ile}$-C-C-2'dA (C).

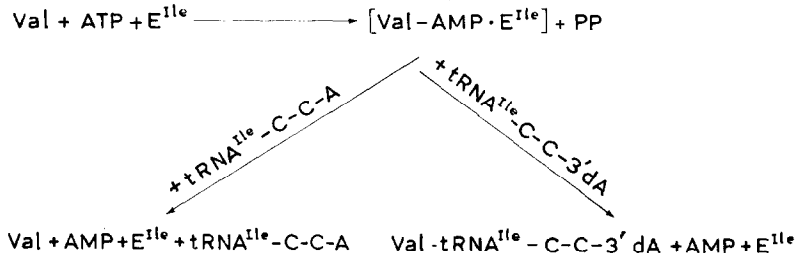
Interestingly, when these modified $tRNA^{Ile}$ species were now tested using valine instead of isoleucine we found that valine was transferred to $tRNA^{Ile}$-C-C-3'dA (B).



2. Materials and methods

All materials used as well as the incorporation of 3'-dAMP into $tRNA^{Ile}$-C-C were essentially the same as described [6].

Isoleucylation and valylation were performed according to [7]. The assay mixture contained 150 mM Tris·HCl buffer pH 7.6, 150 mM KCl, 10 mM $MgSO_4$, 0.06 mM [^{14}C]isoleucine or [^{14}C]valine and 0.003 mM $tRNA^{Ile}$-C-C-N. The amount of isoleucyl-tRNA synthe-tase (EC 6.1.1.5) of specific activity 552 units/mg [8] and valyl-tRNA synthetase (EC 6.1.1.9) of specific activity 276 units/mg [8] is given in table 1. 20 μg of tRNA-nucleotidyl transferase (EC 2.7.7.21) of specific activity 5000 units/mg [9] in the 100 μl assay mixture were used where specified.



3. Results

The results of the aminoacylation experiments are summarized in table 1. From the first line it can be seen that isoleucyl-tRNA synthetase does not transfer valine to $tRNA^{Ile}$-C-C-A. Besides this the experiment shows that there is no [^{14}C]isoleucine in the [^{14}C]valine used. The second line shows that a residual amount of $tRNA^{Val}$-C-C-A in $tRNA^{Ile}$-C-C-A is aminoacylated by valyl-tRNA synthetase. Together with the results from the first line this shows that the isoleucyl-tRNA synthetase used is completely free of valyl-tRNA synthetase. In the third and fourth lines, $tRNA^{Ile}$-C-C, the starting material for the incorporation of 3′-deoxyadenosine, is characterized. Since isoleucylation takes place only in the presence of tRNA-nucleotidyl transferase and ATP, the 3′-terminal AMP must be completely absent. $tRNA^{Val}$ was difficult to separate from $tRNA^{Ile}$ in all procedures tested. Therefore care was taken to keep this impurity as low as possible. As can be seen from the fourth line, the $tRNA^{Val}$ content is about 5% of that of $tRNA^{Ile}$ in the $tRNA^{Ile}$-C-C, whereas it is 13% in the $tRNA^{Ile}$-C-C-A preparation (first and second lines).

The crucial experiment is given in the fifth line. It can be seen that $tRNA^{Ile}$-C-C-3′dA prepared from the

Table 1
Aminoacylation of $tRNA^{Ile}$-C-C-A, $tRNA^{Ile}$-C-C and $tRNA^{Ile}$-C-C-3′dA with valine and isoleucine using isoleucyl-tRNA synthetase and valyl-tRNA synthetase respectively

$tRNA^{Ile}$ tested	Enzyme used	Amount of enzyme used (μg/100 μl)	Isoleucine acceptance (nmol/A_{260} unit tRNA)		Valine acceptance (nmol/A_{260} unit tRNA)	
			−NTase*	+ NTase*	−NTase*	+ NTase*
$tRNA^{Ile}$-C-C-A	Isoleucyl-tRNA synthetase	125	1.47	1.47	0.07	–
	Valyl-tRNA synthetase	100	–	–	0.19	–
$tRNA^{Ile}$-C-C	Isoleucyl-tRNA synthetase	50	0.03	1.44	–	–
	Valyl-tRNA synthetase	100	–	–	–	0.07
$tRNA^{Ile}$-C-C-3′dA	Isoleucyl-tRNA synthetase	125	1.60	1.60	1.53	1.50
	Valyl-tRNA synthetase	500	0.01	–	0.06	–

* tRNA-nucleotidyl transferase.
– Signifies values have not been determined since they are not relevant to the topic under discussion.

$tRNA^{Ile}$-C-C accepts isoleucine as well as valine up to 100% if isoleucyl-tRNA synthetase is used. As shown in the sixth line even a huge amount of valyl-tRNA synthetase neither transfers isoleucine nor valine to $tRNA^{Ile}$-C-C-3′dA. The difference in maximal isoleucine acceptance between $tRNA^{Ile}$-C-C and $tRNA^{Ile}$-C-C-3′dA is due to the general observation that aminoacylation of a tRNA-C-C using the two enzymes tRNA-nucleotidyl transferase and aminoacyl-tRNA synthetase yields slightly lower levels than aminoacylation of a tRNA-C-C-A.

4. Concluding remarks

The data presented here do not allow further speculation about the mechanism which leads to hydrolysis of the Val-AMP-isoleucyl-tRNA synthetase complex. They point, however, to the role played by the 3′-hydroxyl group of the last ribose and offer new possibilities for further investigations on this reaction.

The data are in line with earlier observations in the $tRNA^{Phe}$-C-C-A and phenylalanyl-tRNA synthetase system [10]. There we showed that the 3′-terminal adenosine of $tRNA^{Phe}$-C-C-A triggers the enzyme from a nonreactive to a reactive state during the aminoacylation reaction. From the data given here as well as from indications in the literature (for references see [1]) it may be concluded that triggering of aminoacyl-tRNA synthetases by the 3′end of tRNA – which actually is the reactive center – is not unique for phenylalanyl-tRNA synthetase but is more general. The biological implications of this phenomenon will be discussed in context with further work on this topic.

Acknowledgements

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References

[1] Loftfield, R. B. (1972) The Mechanism of Aminoacylation of tRNA in: Progress in Nucleic Acid Research and Molecular Biology, (Davidson, J. N. and Cohn, W. E. eds) Vol. 12, p. 99 ff. Academic Press, New York, London.
[2] Bergmann, F. H., Berg, P. and Dieckmann, M. (1961) J. Biol. Chem. 236, 1735–1740.
[3] Loftfield, R. B. and Eigner, E. R. (1965) J. Biol. Chem. 240, 1482–1484.
[4] Baldwin, A. N. and Berg, P. (1966) J. Biol. Chem. 241, 839–845.
[5] Arcà, M., Frontali, L., Sapora, O. and Tecce, G. (1967) Biochim. Biophys. Acta 145, 284–291.
[6] Cramer, F., Faulhammer, H., von der Haar, F., Sprinzl, M. and Sternbach, H. (1975) FEBS Letters preceeding paper.
[7] Schlimme, E., von der Haar, F. and Cramer, F. (1969) Z. Naturforschg. 24b, 631–637.
[8] von der Haar, F. (1973) Eur. J. Biochem. 34, 84–90.
[9] Sternbach, H., von der Haar, F., Schlimme, E., Gaertner, E. and Cramer, F. (1971) Eur. J. Biochem. 22, 166–172.
[10] von der Haar, F. and Gaertner, E. (1975) Proc. Natl. Acad. Sci. USA.(1975) 72, 1378–1382.