NUCLEOTIDE SEQUENCE IN $tRNA_{2a}^{Val}$ FROM BAKER'S YEAST

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1. Introduction

Knowledge of the primary structure of isoacceptor tRNAs is important both for understanding the mechanism of their participation in cell metabolism, in general, and for developing the problem of specific recognition of tRNAs by aminoacyl-tRNA synthetases, in particular.

Previously [1] we have separated $tRNA_2^{Val}$ and $tRNA_1^{Val}$ from baker's yeast by chromatography and have shown that $tRNA_2^{Val}$ consists of at least three isoacceptor valine tRNA species. Two of them, $tRNA_{2a}^{Val}$ and $tRNA_{2b}^{Val}$, have been isolated 90% and 70% pure, respectively [1]. Comparative analysis of guanyl-RNase digests of $tRNA_{2a}^{Val}$, $tRNA_{2b}^{Val}$ and $tRNA_1^{Val}$ by using the technique of two-dimensional thin-layer chromatography has revealed considerable structural differences between these isoacceptor tRNA species from *Saccharomyces cerevisiae*.

We have studied the primary structure of $tRNA_{2a}^{Val}$ which is presented in this paper. We compare it with the structure of $tRNA_1^{Val}$ baker's yeast analysed previously in this laboratory [2]*. Twenty-four distinguishing points can be noted in these valine tRNAs. The anticodon region of $tRNA_{2a}^{Val}$ is represented with the sequence NAC (fig. 1) where N corresponds to a uridine analogue nucleoside of unknown structure. There are four triplets codings for the amino acid valine: GUU, GUC, GUA and GUG. GU^A_G are likely to correspond to $tRNA_{2a}^{Val}$.

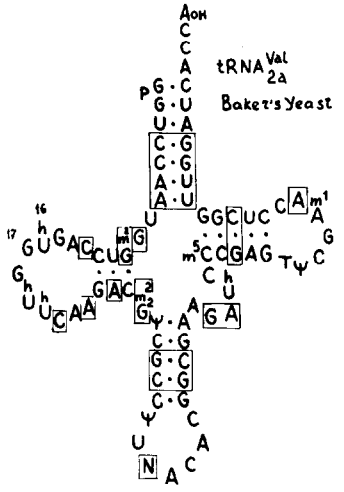

Fig. 1. Primary structure of $tRNA_{2a}^{Val}$ from baker's yeast in the clover leaf form. Regions distinct to the corresponding sites in $tRNA_1^{Val}$ from the same source are in brackets. Moreover, $tRNA_{2a}^{Val}$ lacks the nucleoside C between the positions 16 and 17.

2. Materials and methods

Total tRNA was isolated from baker's yeast by the technique described previously [5]. $tRNA_{2a}^{Val}$ was purified by four chromatographic procedures described in detail in our recent publication [1]: BD-cellulose

* Bonnet et al. have determined the primary structure of $tRNA_{II}^{Val}$ from brewer's yeast [3] and suggested that the structure of $tRNA_1^{Val}$ from baker's yeast [2] contains errors at the positions 47, 49, 50 and 66. This suggestion was proved [4]. The structure of $tRNA_1^{Val}$ from baker's yeast and that of $tRNA_{II}^{Val}$ from brewer's yeast are identical.

(pH 3.5, EDTA), DEAE-Sephadex A-25, BD-cellulose (pH 4.5, Mg^{2+}) and reversed phase chromatography in RPC-3 system.

The products of the complete digestion of $tRNA_{2a}^{Val}$ by guanyl-RNase from *Actinomyces aureoverticillatus* [6] were separated by column chromatography on DEAE-cellulose in 7 M urea at pH 7.4 [7] and rechromatography on the same ion-exchanger in 7 M urea at pH 3.4 [8]. Digestion products of $tRNA_{2a}^{Val}$ formed after treatment with pancreatic ribonuclease were separated by column chromatography on DEAE-cellulose in 7 M urea (pH 7.4) and on Dowex-1X4 in the formate system [9]. Base sequences in oligonucleotides were established by analysing the products of complete and incomplete digestions of those in the presence of exonuclease A-5 from *Actinomyces aureoverticillatus* [10] and diesterase from snake venom. For the same purpose, complete digests of oligonucleotides by pancreatic or guanyl-RNases were used as well. To determine the base composition, oligonucleotides were treated with T_2 RNase and alkaline phosphatase from *E. coli*. Separation of the products formed after treatment with diesterases, ribonucleases or phosphatase was performed by thin layer chromatography on cellulose placed on quartz plates. To increase the sensitivity of the technique, a glass plate covered with a luminophore was placed on a dried chromatogram. UV-spectra of the products eluted from the spots were registered in 0.2 ml cuvettes at a length of the optical path of 4 cm. Halves and fragments of $tRNA_{2a}^{Val}$ were obtained by treatment with pancreatic RNase at 0°C in the presence of Mg^{2+} ions and separated by chromatography on DEAE-cellulose in 7 M urea at pH 3.4. The procedures mentioned above will be described in detail elsewhere.

3. Results

Oligonucleotides obtained after complete degradation of $tRNA_{2a}^{Val}$ by guanyl-RNase and pancreatic ribonuclease are shown in table 1. $tRNA_{2a}^{Val}$ was not cleaved by guanyl-RNase at 0°C in the presence of Mg^{2+} ions (0.01 M) even if the amount and concentration of the enzyme was 10 times as high as that necessary for cleavage of $tRNA_1^{Val}$ into halves under the same conditions. Halves and shorter fragments of

Table 1
Products formed by complete degradation of $tRNA_{2a}^{Val}$

With pancreatic RNase	With Guanyl-RNase
C_{OH}, $13C_p$, $3U_p$, $2\psi_p$, hUp, m^5Cp, Np	m^2Gp, 5Gp
	pGp
3(A–Cp)	A–Gp
G–Cp	C–Gp
$m^2G-\psi p$	hU–Gp
A–A–Up	U–U–Gp
A–G–hUp	A–A–Gp
G–G–Cp	ψ–C–Gp
G–G–Up	A–C–m_2^2Gp
G–G–hUp	T–ψ–C–Gp
G–m_2^2G–Up	U–C–C–A–Gp
pG–G–Up	A–hU–C–m^5C–C–Gp
G–A–G–Tp	A–U–C–A–C–C_{OH}
G–G–A–Up	m^1A–A–C–C–U–C–Gp
G–m^1A–A–Cp	hU–hU–C–A–A–Gp
A–A–G–A–Cp	U–C–C–A–A–U–Gp
G–A–A–G–A–hUp	C–C–ψ–U–N–A–C–A–C–Gp

$tRNA_{2a}^{Val}$ were obtained by treating with pancreatic ribonuclease at 0°C in the presence of Mg^{2+} ions (0.05–0.1 M). On the basis of the data obtained the primary structure of $tRNA_{2a}^{Val}$ was reconstituted, it is shown as a clover-leaf model in fig. 1.

A nucleotide possessing the properties of N was not present in neither of the tRNA base sequences known to date. The UV-spectrum of N resembles that of thymidine (fig. 2) but it cannot be identified as

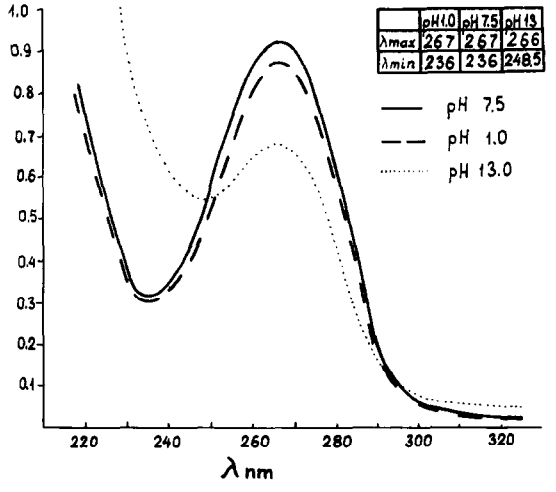

Fig. 2. UV-spectrum of the odd nucleotide N. The spectrum was read in a Hitachi Model EPS-3T. Optical path of 1 cm.

thymidine since it shows quite different mobility in several thin layer chromatography systems which is most frequently close to the mobility of ψ. Unfortunately, we have not enough material to study the chemical structure of N which is of extreme interest since N is a part of the $tRNA_{2a}^{Val}$ anticodon. $tRNA_{2a}^{Val}$ contains 12 odd nucleotides: 3ψ, 4hU, m^5C, m^1A, m_2^2G, m^2G,N. The three latter ones lack $tRNA_1^{Val}$. On the other hand, $tRNA_{2a}^{Val}$ lacks m^1G and m^7G which are present in $tRNA_1^{Val}$.

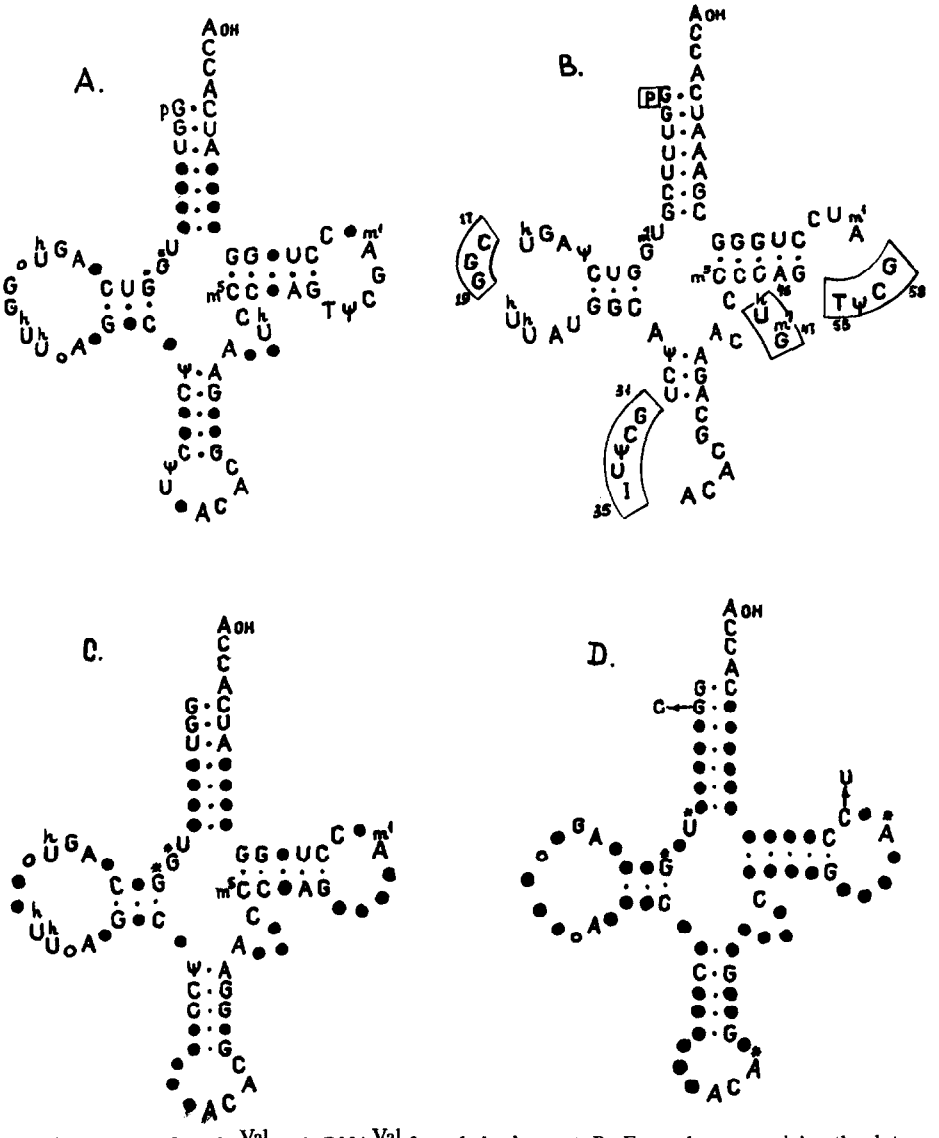

Fig. 3. A–Generalized structure of $tRNA_{2a}^{Val}$ and $tRNA_1^{Val}$ from baker's yeast. B–Formula summarizing the data on excision of fragments from $tRNA_1^{Val}$ molecule. In brackets are the regions which can be excised without affecting the valine acceptor ability. Following percentages of activity are restored after excision of respective fragments: (31–35) – 73% [13], (17–19) – 92%; (47–48) – 83%; (55–58) – 35% [14]. C–Formula summarizing the results of comparison of formulas presented in figs. 3A and 3B. D–Generalized structure obtained by comparison of the formula C with $tRNA_1^{Val}$ from *E. coli* [16]. Letters at the head of the arrows denote nucleotide occupying the corresponding positions in the molecules of $tRNA_{2a}^{Val}$ and $tRNA_{2b}^{Val}$ from *E. coli* [17]. Full circles (•) denote distinctive nucleotides, open circles (○) designate the lack of a nucleotide at a corresponding position.

4. Discussion

The data obtained by Lagerqvist et al. [11,12] indicate that $tRNA_1^{Val}$ and $tRNA_2^{Val}$ from baker's yeast are acylated by one valyl tRNA synthetase. Therefore it is reasonable to conclude that the sites responsible for the specific recognition are most probably localized in the overlapping regions, rather than in the distinctive ones. On the other hand, to date it is not known which of two factors plays the leading role in specific fitting between tRNA and cognate ligase: interaction between certain chemical groups or steric fit of the partners. Nevertheless, the comparative analysis of primary structures of isoacceptor tRNAs seems to be not useless. Fig. 3A shows generalized structure of $tRNA_1^{Val}$ and $tRNA_{2a}^{Val}$ from baker's yeast, obtained by exclusion of distinctive regions. This kind of information can be supplemented with the data obtained in the experiments with incomplete molecules of $tRNA_1^{Val}$ from baker's yeast [13,14]. Fig. 3b shows the regions which being split off from the molecule due to enzymatic or chemical treatment do not affect to acceptor activity of $tRNA_1^{Val}$. Fig. 3C summarizes the information presented in two preceding pictures.

Valyl tRNA synthetase from baker's yeast is able to charge $tRNA^{Val}$ from *E. coli* [15] with the same yields as the corresponding *E. coli* enzymes. The comparison of the structures of tRNAs from yeast and that of *E. coli* may be of interest. Fig. 3D shows comparative analysis of the generalized structure shown in fig. 3C and *E. coli* $tRNA_1^{Val}$. $tRNA_1^{Val}$ [16] and $tRNA_{2A,B}^{Val}$ [17] from *E. coli* contain 22 distinctive positions. It might seem that a comparison the generalized structure, shown in fig. 3D with $tRNA_{2A,B}^{Val}$ from *E. coli* would lead to a decrease of the number of common nucleotides. It is interesting to note that it is not the case: only two additional positions are excluded from common regions (fig. 3D).

The formula obtained does not contradict the suggestion that tRNAs contain a 'discriminator site' [18]. This formula has much in common with the generalized scheme deduced from the data of incorrect charge catalyzed by yeast valyl tRNA synthetase [19].

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