PRIMARY STRUCTURE OF $tRNA_2^{Val}$ FROM BREWER'S YEAST

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1. Introduction

In order to study the interactions between valyl-tRNA synthetase of yeast and the corresponding tRNAs, we have isolated $tRNA_2^{Val}$ from brewer's yeast [1]. We have studied its degradation products obtained by pancreatic and T_1 ribonuclease digestions. We expected to find the mono- and oligonucleotides described by Bayev et al. [2] after ribonuclease digestions of $tRNA_1^{Val}$ from baker's yeast, but observed several differences, which could be explained by building a nucleotide sequence analogous to that described by Bayev et al., but differing at four points.

2. Methods

Commercial brewer's yeast tRNA (Boehringer) was first subjected to countercurrent distribution in Holley's phosphate–isopropanol–formamide solvent system [3] as previously described [4].

The assay for ^{14}C-valine accepting activity performed as reported elsewhere [1] shows three peaks [1]: $tRNA_1^{Val}$, $tRNA_2^{Val}$, $tRNA_3^{Val}$; $tRNA_1^{Val}$ has the lowest solubility in the organic phase. The major component is $tRNA_2^{Val}$, which corresponds to the $tRNA_1^{Val}$ of Bayev et al. [5] and was obtained by countercurrent distribution under the same conditions as we use. However Bayev et al. [5] showed the existence of a $tRNA^{Val}$ less soluble in the organic phase than their $tRNA_1^{Val}$ We confirmed the existence of this tRNA in brewer's yeast [1] and prefer therefore to call the second $tRNA^{Val}$ peak in the countercurrent distribution $tRNA_2^{Val}$, although it was called $tRNA_1^{Val}$ by Bayev et al. [5].

$tRNA_2^{Val}$ was further purified either by reversed phase chromatography [1] according to Kelmers et al. [6] or by chromatography on BD-cellulose at pH 3.5 according to Gillam et al. [7] (fig. 1).

Structural analysis was accomplished by enzymatic fragmentation with subsequent resolution of the fragments by chromatography or electrophoresis. The methods for complete hydrolysis by T_1 or pancreatic ribonucleases, subsequent separation of the oligo-

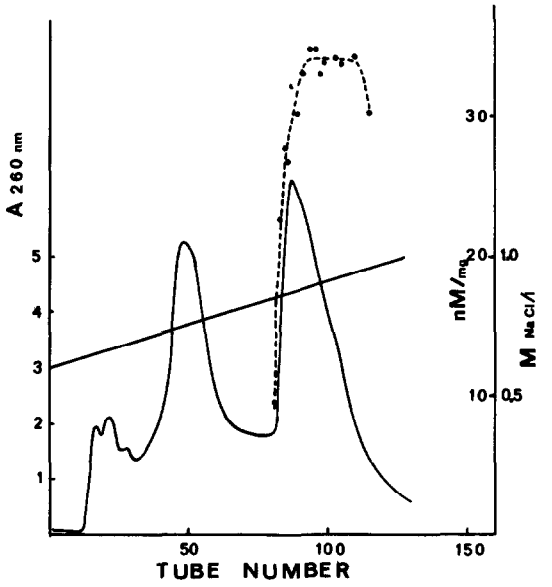

Fig. 1. Chromatography on BD-cellulose (2 cm × 120 cm) of fractions enriched in $tRNA_2^{Val}$ by countercurrent distribution. 225 mg tRNA were charged on the column. Elution by a linear gradient of NaCl 0.6–1 M in 5×10^{-3} M formate buffer pH 3.5, 5×10^{-3} M EDTA. Total volume of the gradient 3,000 ml. Volume of the fractions 2.4 ml. —— absorbance at 260 nm; - - - - - accepting activity expressed in nmoles of ^{14}C valine/mg tRNA.

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nucleotides, partial digestion with snake venom phosphodiesterase, alkaline hydrolysis of oligonucleotides followed by separation of mononucleotides by thin-layer chromatography have been already described by Gangloff et al. [8].

Acidic hydrolysis of oligonucleotides was performed in HClN for 1 hr at 100°.

3. Results

3.1. *Hydrolysis by T_1 ribonuclease*

The products of complete T_1 ribonuclease digests were separated by using DEAE-cellulose column chromatography. Fig. 2 shows such a separation. The fractions corresponding to the peaks 10, 11 and 12 were pooled and submitted to a further separation by chromatography on DEAE-cellulose at pH 3.0. The same procedure was used with the fractions corresponding to peak 13.

The base composition was determined by alkaline hydrolysis for all the isolated oligonucleotides.

Table 1 lists the composition of all the oligonucleotides obtained. Our data are completely consistent with the results of Bayev et al. [2], except for the amount of G (about 6 instead of 7) and except for the structure of oligonucleotide 15.

Among the alkaline hydrolysis products of oligonucleotide 15, a degradation product was found whose

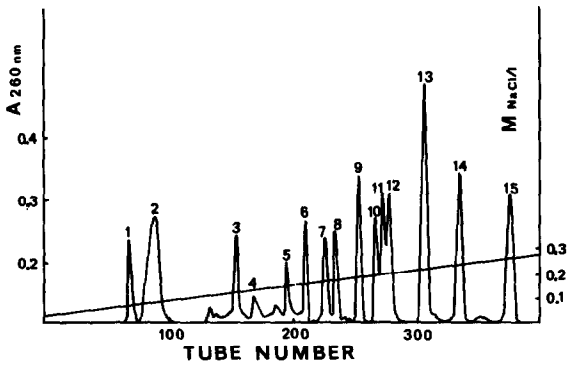

Fig. 2. Chromatography of 44 A units of a T_1 ribonuclease digest of brewer's yeast $tRNA_2^{Val}$ on DEAE-cellulose (210 cm × 0.5 cm). Elution by a linear gradient of NaCl 0.015–0.3 M in 2 × 10^{-2} M. Tris-HCl buffer pH 7.3, 7 M urea. Total volume of the gradient 1,200 ml. Volume of the fractions 3 ml.

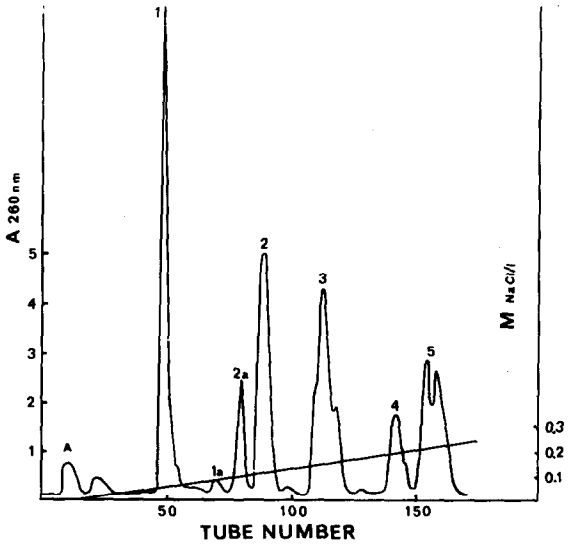

Fig. 3. Chromatography of a pancreatic ribonuclease digest of 10 mg of brewer's yeast $tRNA_2^{Val}$ on DEAE-cellulose (70 cm × 0.5 cm). Elution by a linear gradient of NaCl 0–0.24 M in 2 × 10^{-2} M. Tris-HCl buffer pH 7.4, 7 M urea. Total volume of the gradient 360 ml. Volume of the fractions: 2.4 ml.

spectral characteristics were identical to that of 2,4-diamino-6-hydroxy-5-*N*-methyl formamido pyrimidine given by 7 MeG after alkaline treatment. The existence of 7 MeG was verified by an acidic hydrolysis of peak 15. A digestion with pancreatic ribonuclease gave A–A-Cp, 7 MeG–hUp, A–Gp, 5 MeCp and Cp. After removal of the 3′-terminal phosphate by phosphomonoesterase two samples of peak 15 (15 A units) dissolved in 2 ml of triethylammonium bicarbonate buffer 0.05 M pH 8.5, $MgCl_2$ 0.05 M, were submitted to a partial hydrolysis by 100 μg of venom phosphodiesterase at 37° respectively for 15 min and for 33 min. This treatment gave mixtures of oligonucleotides which were chromatographed together on DEAE-cellulose. 7 peaks were obtained. Table 2 gives the results of the analyses of these peaks. These results led to the following sequence:

A–A–C–7 MeG–hU–C–5 MeC–C–C–A–Gp

It differs from the sequence proposed by Bayev et al. [2] for the corresponding oligonucleotide obtained from $tRNA_1^{Val}$ from baker's yeast:

Table 1
Composition and molar ratios of nucleotides and oligonucleotides obtained by T_1 digestion of $tRNA_2^{Val}$ of brewer's yeast compared with the results described by Bayev et al. [2] for $tRNA_1^{Val}$ from baker's yeast.

Peak	Composition		Molar ratios	
	According to Bayev et al. [2]	Observed	According to Bayev et al. [2]	Observed
1	U–1 MeG > p	U–1 MeG > p	1	0.99
2	Gp	Gp	7	5.6
3	C–Gp	C–Gp	1	1.32
4	–	A–Gp + A–Ap + (A, C)p + (A, U)p	–	0.52
5	hU–C–Gp	(hU, C)Gp	1	1.13
6	C–A–Gp	(C, A)Gp	1	1.21
7	C–Ψ–U–Ip	(C, Ψ, U)Ip	1	0.83
7a	pGp	pGp	1	0.83
8	T–Ψ–C–Gp	(T, Ψ, C)Gp	1	0.96
9	1 MeA–U–C–C–U–Gp	(1 MeA, U_2, C_2)Gp	1	0.85
10	hU-hU–A–U–Gp	hU–hU–A–U–Gp	1	1.17
11	U–U–U–C–Gp	(U_3, C)Gp	1	0.82
12	U–C–Ψ–A–Gp	(U, C, Ψ, A)Gp	1	0.98
13a	A–C–A–C–Gp	(A_2, C_2)Gp	1	0.79
13b	C–A–Ψ–C–U–Gp	(A, C_2, U, Ψ)Gp	1	1.13
14	A–A–A–U–C–A–C–C–A	(A_4, C_3, U)A	1	0.80
15	A–A–C–hU–5 MeC–C–C–C–A–Gp	A–A–C–7 MeG–hU–C–5 MeC–C–C–A–Gp	1	0.75

A–A–C–hU–5 MeC–C–C–C–A–Gp.

3.2. *Hydrolysis by pancreatic ribonuclease*

Total pancreatic ribonuclease digests were chromatographed on DEAE-cellulose (fig. 3). The oligonucleotides present in the peaks 1 to 5 were purified by high voltage electrophoresis or by chromatography on DEAE-cellulose at pH 3.0.

Table 3 lists the composition of all the fragments formed by this digestion. There is an inconsistency between the data presented by Bayev et al. [2] and ours. The DEAE-cellulose chromatography shows a tetranucleotide peak (peak 4) which yields after alkaline hydrolysis 3.2 Gp and 1 Cp. After desalting, this product was submitted to an electrophoresis with a mixture of G–G–G–Cp and A–G–A–Cp derived from a pancreatic digestion of $tRNA^{Asp}$ which contains these two tetranucleotides [9]. Fig. 4 shows that the tetranucleotide obtained from $tRNA_2^{Val}$ migrated at the same place as G–G–G–Cp. This product was not obtained by Bayev et al. [2]. However, we did not find the pentanucleotide G–G–G–G–Cp described by Bayev et al. in the peak 5 of fig. 3.

Table 2
Analyses of the oligonucleotides produced by partial phosphodiesterase digestion of peak 15.

Peak	Products of alkaline hydrolysis	Molar ratios	Structures
1	Ap, Cp, 7 MeGp, hU_{OH}	2.0:1.02:1.2 :0.6	(A–A–C–7 MeG)hU_{OH}
2	Ap, Cp, 7 MeGp, hUp, C_{OH}	2.0:1.0 :0.7 :0.7:1.04	(A–A–C–7 MeG)hU–C_{OH}
3	Ap, Cp, 7 MeGp, hUp, 5 MeC_{OH}	2.0:2.02:1.38:0.8:1.08	(A–A–C–7 MeG)hU–C–5 MeC_{OH}
4	Ap, Cp, 7 MeGp, hUp, 5 MeCp, C_{OH}	2.0:2.09:0.76:0.6:0.95:1.05	(A–A–C–7 MeG)hU–C–5 MeC–C_{OH}
5	Ap, Cp, 7 MeGp, hUp, 5 MeCp. C_{OH}	2.0:3.29:0.80:0.7:0.91:1.06	(A–A–C–7 MeG)hU–C–5 MeC–C–C_{OH}
6	Ap, Cp, 7 MeGp, hUp, 5 MeCp, A_{OH}	2.0:4.3 :0.5 :0.8:0.8 :0.7	(A–A–C–7 MeG)hU–C–5 MeC–C–C–A_{OH}
7	Ap, Cp, 7 MeGp, hUp, 5 MeCp, G_{OH}	3.0:4.1 :0.9 :0.8:0.9 :1.05	(A–A–C–7 MeG)hU–C–5 MeC–C–C–A–G_{OH}

Table 3
Composition and molar ratios of nucleosides, nucleotides and oligonucleotides obtained by pancreatic ribonuclease of $tRNA_2^{Val}$ from brewer's yeast compared with the results described by Bayev et al. [2] for $tRNA_1^{Val}$ from baker's yeast.

Peak	Composition		Molar ratios	
	According to Bayev et al. [2]	Observed	According to Bayev et al. [2]	Observed
Adenosine	A	A	1	1.10
1	Cp	Cp	12	11.94
	5 MeCp	5 MeCp	1	1.20
	Up	Up	5	5.15
	Ψp	Ψp	3	2.10*
	hUp	hUp	2	1.00
1a	–	7 MeG–hUp	0	1.30
2a	G–1 MeA–Up	(G, 1 MeA)Up	1	1.39
2A	G–Up	G–Up	1	0.98
B	A–Up	A–Up	1	1.03
C	A–Ψp	A–Ψp	1	0.92
D	G–Cp	G–Cp	2	1.85
E	A–Cp	A–Cp	2	1.61
3A	G–G–hUp	G–G–hUp	1	0.81
B	1 MeG–G–Up	(1 MeG, G)Up	1	0.71
C	A–G–Tp	(A, G)Tp	1	0.93
D	A–G–hUp	(A, G)hUp	1	0.95
E	G–G–Cp	G–G–Cp	1	0.96
F	I–A–Cp	(I, A)Cp	1	0.76
4	–	G–G–G–Cp	0	1.07
5	G–G–G–G–Cp	–	1	0
A	pG–G–Up	pG–G–Up	1	0.71
B	A–G–A–A–Cp	(A_3, G)Cp	1	1.14
C	G–A–A–A–Up	(A_3, G)Up	1	0.88

* Values for Ψp were systematically too low. For discussion see [8].

4. Discussion

The results of the investigation on the primary sequence of $tRNA_2^{Val}$ of brewer's yeast lead to the following conclusions: the sequence of brewer's yeast $tRNA_2^{Val}$ differs from the sequence proposed by Bayev et al. [2] for baker's yeast $tRNA_1^{Val}$ at four points: there is a supplementary 7 MeG at position 47 from the 5′-terminal end, but no G between the stem containing the TΨC loop and the stem (position 12 from the 3′-terminal end). In fact the presence of G between the two stems was rather strange, as no nucleotide has been found there in any other tRNA of known structure. Moreover there is an inversion in the position of 5 MeG and C in the extra-loop. We found the sequence, hU–C–5 MeC, instead of the sequence, hU–5 MeC–C. These results led us to propose the following structure of $tRNA_2^{Val}$ from brewer's yeast (fig. 5).

The sequence A–C–7 MeG–hU–C–5 MeC that we found for the region of the extra-loop, looks very similar to the sequences of the extra loops of $tRNA^{Phe}$ (yeast) A–G–7 MeG–U–C–5 MeC [10], of $tRNA^{Phe}$ (wheat germ) A–G–7 MeG–hU–C–G [11] and of $tRNA^{Val}$ (*E. coli*) G–G–7 MeG–U–C–G [12]. $tRNA^{Met}$ and $tRNA^{FMet}$ (*E. coli*) also have similar structures in the extra-loop. It is surprising that $tRNA^{Val}$ of baker's yeast [2] and that of Torula

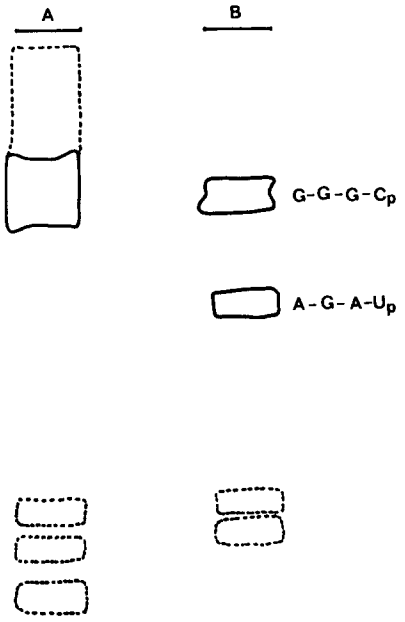

Fig. 4. High voltage electrophoresis of peak 4 in fig. 3 in 7% HCOOH at 1,000 V for 12 hr in conditions described in [14]. A = peak 4; B = mixture of G–G–G–Cp and A–G–A–Up.

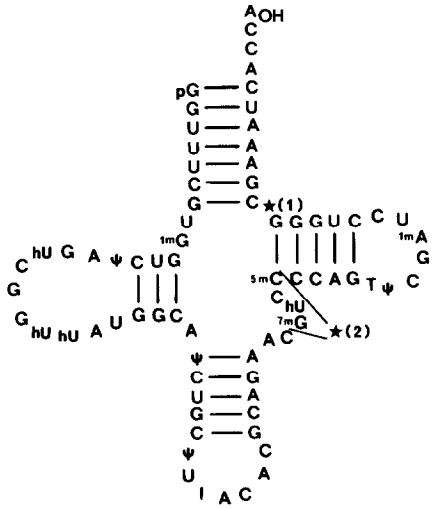

Fig. 5. Structure of $tRNA_2^{Val}$ from brewer's yeast. According to Bayev et al. [2] an additional G in*(1) and sequence hU–5 MeC–C in position* (2).

[13] do not show a sequence 7 MeG–hU or 7 MeG–U.

Reinvestigation of the structure of $tRNA_1^{Val}$ from baker's yeast will be performed in collaboration with Dr. Bayev.

Acknowledgements

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References

[1] J. Bonnet, G. Dirheimer and J.P. Ebel, Bull. Soc. Chim. Biol. 51 (1969) 415.
[2] A.A. Bayev, T.V. Venkstern, A.D. Mirzabekov, A.I. Krutilina, L. Li and V.D. Axelrod, Molecular Biol. Moscow 1 (1967) 754. English edition p. 631.
[3] R.W. Holley, J. Apgar, G.A. Everett, J.T. Madison, S.H. Merrill and A. Zamir, Cold Spring Harbor Symp. Quant. Biol. 28 (1963) 117.
[4] G. Dirheimer and J.P. Ebel, Bull. Soc. Chim. Biol. 49 (1967) 1679.
[5] A.A. Bayev, T.V. Venkstern, A.D. Mirzabekov, A.I. Krutilina, V.D. Axelrod, L. Li and V.A. Engelhardt, in: Genetic Elements (Academic Press, London, 1966) p. 287.
[6] A.D. Kelmers, G.D. Novelli and M.P. Stulberg, J. Biol. Chem. 240 (1965) 3979.
[7] I. Gillam, S. Millward, D. Blew, M. von Tigerström, E. Wimmer and G.M. Tener, Biochemistry 6 (1967) 3043.
[8] J. Gangloff, G. Keith, J.P. Ebel and G. Dirheimer, Biochim. Biophys. Acta (1971) submitted for publication.
[9] G. Keith, J. Gangloff, J.P. Ebel and G. Dirheimer, Compt. Rend. 271 (1970) 613.
[10] U.L. Rajbhandary, S.H. Chang, A. Stuart, R.D. Faulkner, R.M. Hoskinson and H,G. Khorana, Proc. Natl. Acad. Sci. U.S. 57 (1967) 751.
[11] B.S. Dubock, G. Katz, E.K. Taylor and R.W. Holley, Proc. Natl. Acad. Sci. U.S. 62 (1969) 941.
[12] M. Yaniv and B.G. Barrell, Nature 222 (1969) 278.
[13] T. Mizutani, M. Miyazaki and S. Takemura, J. Biochem. (Tokyo) 64 (1968) 839.
[14] J. Gangloff, G. Keith and G. Dirheimer, Bull. Soc. Chim. Biol. 52 (1970) 125.