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# Primary structure of baker's yeast $tRNA_{2b}^{Val}$

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## ABSTRACT

The minor form of valine tRNA from baker's yeast - $tRNA_{2b}^{Val}$ - purified by column chromatography was completely digested with guanylo-RNase and pancreatic RNase. The products of these digestions were separated by a combination of thin-layer chromatography on cellulose and high voltage electrophoresis on DEAE-paper and then identified. The halves of $tRNA_{2b}^{Val}$ were prepared by partial digestion with pancreatic RNase and their complete guanylo-RNase and pancreatic RNase, digests were analysed. Basing on the obtained data the primary structure of baker's yeast $tRNA_{2b}^{Val}$ was reconstructed.

## INTRODUCTION

Earlier two minor forms of valine tRNA-$tRNA_{2b}^{Val}$ and $tRNA_{2b}^{Val}$ in addition to the major $tRNA_{1}^{Val}$ have been found in baker's yeast [1]. One of them, $tRNA_{2a}^{Val}$, was isolated and its nucleotide sequence was established [2]. In this paper we describe the purification of $tRNA_{2b}^{Val}$, presented in the crude baker's yeast tRNA in amount of 0.2-0.4%, and the procedure of its sequencing.

## MATERIALS AND METHODS

tRNA, Enzymes and other materials. Baker's yeast $tRNA_{2b}^{Val}$ about 6% pure was isolated by chromatography on BD-cellulose and DEAE-Sephadex as reported by Kryukov et al. [1].

Guanylo-RNase from Actinomyces aureoverticillatus was prepared according to Tatarskaya, et al. [3], $T_2$RNase from Taka-Diastase was obtained by modified method of Ruzhizky, et al. [4]. Snake venom phosphodiesterase was extracted from qurza venom as described by Nikolskaya, et al. [5] with additional chromatography on SE-cellulose according to Vasilenko

[6]. Pancreatic RNase kindly provided by Dr.Yu.Lebedev was homogeneous by chromatography on Bio-Rex 70. Exonuclease A-5 [7], endonuclease A-236 [8]from Actinomyces sp. and E.coli alkaline phosphatase were a gift of Dr.R.I.Tatarskaya and her collaborators.

BD-cellulose was prepared by the method of Gillam, et al. [9], RPC-5 system by the method of Pearson, et al. [10], hydroxyapatite by the method of Levin [11]. Bentonite was obtained by modified method of Fraenkel-Conrat, et al. [12]. DE81 paper was purchased from Whatman. Cellulose FND for thin-layer chromatography was from Filtrak (GDR) and Vistec D-1 was from Koch-Light Laboratories Ltd. DEAE-cellulose for column chromatography (0.7 mequiv/g) was a product of Olaine (USSR).

Conditions for enzyme degradation. Complete guanylo-RNase digest was obtained by incubation of 1 mg $tRNA^{Val}_{2b}$ in 0.65 ml 40 mM ammonium bicarbonate (pH=7.9)-1mM EDTA with 1500 units of activity of guanylo-RNase at 37°C for 7 hr.

Complete pancreatic RNase digestion was performed by incubation 0.5 mg $tRNA^{Val}_{2b}$ in 0.1 ml 40 mM ammonium bicarbonate (pH=7.9)-1 mM EDTA with 17 μg pancreatic RNase at 37°C for 3 hr.

Cyclophosphates were cleaved in 4 M formic acid at 20-22°C for 3 hr. [12].

The halves of $tRNA^{Val}_{2b}$ were prepared by incubation of 0.46 mg of intact molecules in 0.7 ml 10 mM tris-HCl (pH=7.5)-100 mM magnesium acetate with 0.55 μg pancreatic RNase at 0°C for 5 hr. Reaction was stopped by 10 times dilution of mixture with cold water and by addition of bentonite suspension (0.46 mg bentonite). Bentonite was then removed after centrifugation at 6000 rpm for 20 min.

The composition of the guanylo-RNase digestion products was determined by $T_2$-RNase and pancreatic RNase hydrolysis as described by Kryukov, et al. [13]. In order to determine the composition of pancreatic RNase digestion products 10 μg of each oligonucleotide was incubated with $T_2$ RNase and guanylo-RNase (10 and 4 units of activity, respectively) in 5 mM potassium acetate (pH=4.5) at 37°C for 16 hr.

Dephosphorylation of nucleotides, digestion of oligonucleotides by exonuclease A-5 and snake venom phosphodiesterase (SVPD) [14] were carried out according to Kryukov, et al. [13]. The guanylo-RNase and pancreatic RNase digestion of oligonucleotides were performed as described above for the intact tRNA but in the presence of alkaline phosphatase (4 units of activity per 10 μg of oligonucleotide) in 40 mM ammonium bicarbonate (pH=8.7) for 16 hr.

Digestion with endonuclease A-236 8 was carried out in 15 μl 20 mM tris-HCl (pH=8.0) - 0.5 mM magnesium acetate (1 unit of activity per 1 μg of substrate) at 37°C for 2 hr.

Separation procedures. We used the combination of thin-layer chromatography (TLC) on cellulose in isobutyric acid - 0.5 M $NH_4OH$, pH=3.7 (5:3, v/v) and high voltage electrophoresis on DE81 paper according to Sanger, et al. [15] for separation of guanylo-RNase and pancreatic RNase digests. The electrophoresis was performed at 4000 V on the cooling plate in formic acid pH=1.7 in order to separate the pancreatic RNase digestion products of tRNA and its halves and at pH=1.55 for separation of guanylo-RNase digestion products. This procedure was reported previously [16].

The UV-absorbing spots were eluted by 30% (v/v) triethylammonium bicarbonate (pH=8.0) as described by Gangloff, et al. [17]. The elution was repeated twice.

The halves of $tRNA^{Val}_{2b}$ were separated by column chromatography on DEAE-cellulose in 7M urea (pH=3.4) with linear gradient of NaCl [18,19] and then desalted according to Delihas and Staehelin [20] but using Vistec D-1 and 30% triethylammonium bicarbonate (pH=8.0).

The following solvent systems were used for TLC: A, 1-butanol, water saturated; B, isobutyric acid - 0.5 M $NH_4OH$, pH=3.7 (5:3, v/v); C, 1-butanol-2-propanol- 7.5 M $NH_4OH$ (3:3:2, v/v); D, tert. amyl alcohol - methyl ethyl ketone - water - conc. HCOOH (2:2:1:0.1, v/v), E, tert. butanol - 0.075 M HCOOH, pH=4.8 (1:1, v/v). All solvents used were freshly distilled.

The composition analysis of oligonucleotides was performed by two-dimensional TLC of nucleosides either in systems A and B or in systems C and D [21]. Nucleosides were identified by

their chromatographic mobilities compared with markers and by their UV-spectra at pH=1 and pH=13.

The fragments obtained from digestion of oligonucleotides were subjected to TLC in systems B and E. If these fragments were produced by partial digestion of SVPD or exonuclease A-5, they were degraded with $T_2$RNase and chromatographed in systems A and B. Before developping in the second dimension (system B) the markers were applied to the plate and the derivatives of the fragment were identified by their chromatographic behaviour. The component which moved in system A represented a 3'-end nucleoside of the fragment.

The products of a partial pancreatic digestion of oligonucleotides were analysed in nucleoside form (see above).

Dihydrouridilic acid was identified after elution of the area corresponding to Up by its characteristic loss of absorption in alkaline medium [22].

## RESULTS

Isolation of baker's yeast $tRNA^{Val}_{2b}$. We used two consecutive column chromatographies to purify valine $tRNA_{2b}$. First 6% pure $tRNA^{Val}_{2b}$ was fractionated on hydroxyapatite in the system of Pearson and Kelmers [23] followed by reverse phase chromatography system (RPC-5) according to Pearson, et al. [10]. Finally obtained $tRNA^{Val}_{2b}$ demonstrated complete coincidence of the UV-absorbing peak with the peak of $^{14}$C-valine acceptor activity. The further criteria for the purity of tRNA were the patterns of its guanylo-RNase digests by TLC on cellulose in systems B and E [24] and microcolumn chromatography on DEAE-cellulose (Fig.1). These patterns showed the preparation to be good for the studying of its primary structure.

Guanylo-RNase digest. Valine $tRNA_{2b}$ was digested with guanylo-RNase and the products were separated by combination of TLC and electrophoresis (Fig.2). Mono-, di- and trinucleotides were identified by the combined results of their composition and pancreatic RNase digestion (Table 1). In the same way the structure of pentanucleotide CpApApApGp was established. The analysis of the other oligonucleotides required additional approaches.

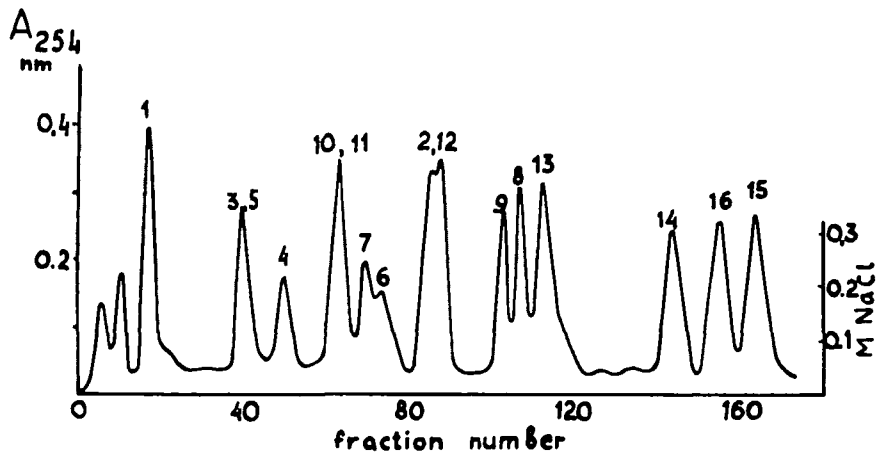



Fig.1. The separation of a complete guanylo-RNase digest of baker's yeast $tRNA^{Val}_{2b}$ on DEAE-cellulose in 7M urea, 0.005 M $KH_2PO_4$, pH=7.4 with a linear gradient of NaCl (0 to 0.3 M). Column 0.25 ml (h=17 cm), elution volume 6 ml, flow rate 0.11 ml/hour. The loading 10 µg [25]. Numbers of the peaks correspond to spots in Fig.2.

Oligonucleotide t2. It had to be the 3'-end of the tRNA as judged by the absence of G in its nucleoside composition. Exonuclease A-5 digestion allowed to determine the 5'-end of t2-ApA and with the results of pancreatic RNase digestion we obtained the sequence $ApApCpApCpC_{OH}$.

Oligonucleotide t9. It had a blue fluorescence due to the presence of $m^7G$, that reduced its UV-absorption when photographing. Dihydrouridine was found during the analysis of the pancreatic RNase digestion product of t9, contained $m^7G$. The complete digestion of t9 with SVPD showed $m^7G$ to be the 5'-end of the oligonucleotide. The partial digestion with the same enzyme gave fragments ($m^7Gp$, Dp, Cp)$m^5C$ and ($m^7Gp$, Dp, Cp, $m^5Cp$)C, and being combined with data of the pancreatic RNase digestion led to the sequence $m^7GpDpCpm^5CpCpGp$.

Oligonucleotide t12. Exonuclease A-5 digestion gave dinucleosidemonophosphate TpΨ, that with data showed in Table 1 allowed to determine the sequence of t12 as TpΨpCpGp.

Oligonucleotide t13. The bound $m^1A$-U in t13 resistant to the $T_2$ RNase action [26] was splitted by SVPD. The complete SVPD digestion indicated $m^1A$ as 5'-end of t13 and the parti-

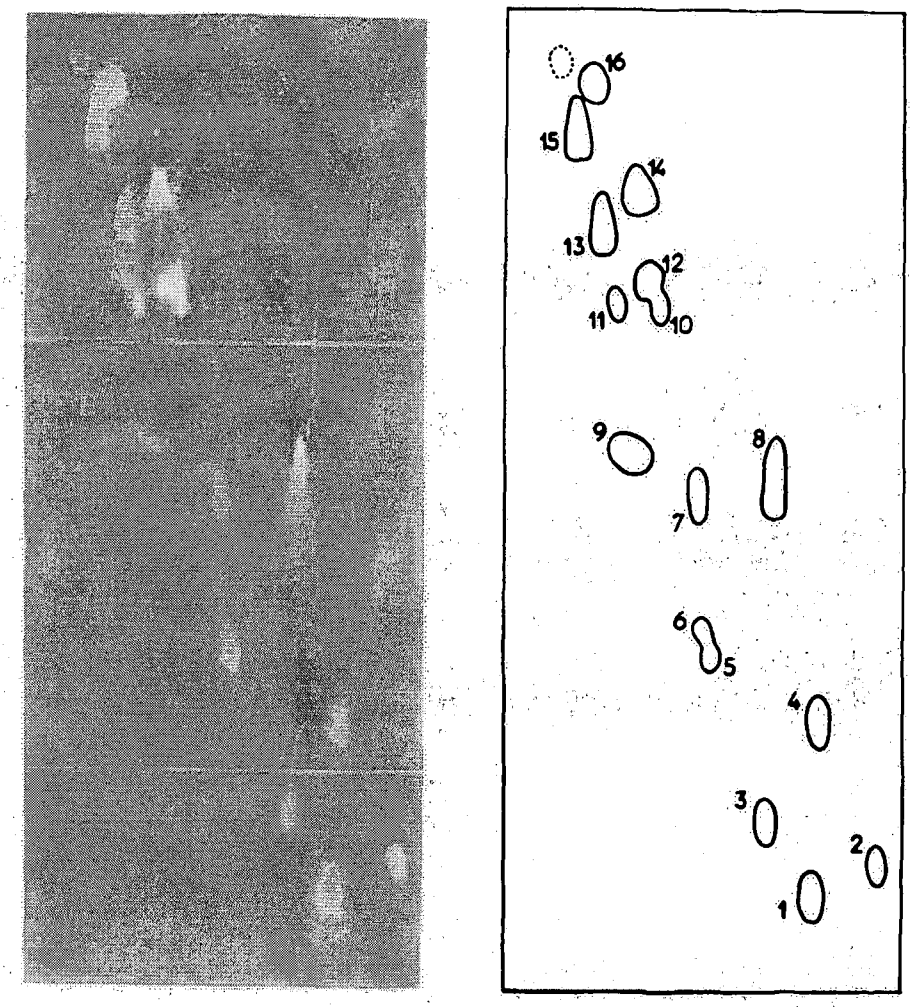



Fig.2. Fingerprint of a complete guanylo-RNase digest of baker's yeast $tRNA^{Val}_{2p}$. First dimension, left to right, TLC on cellulose in system B. Second dimension, top to bottom, electrophoresis on DEAE-paper at pH=1.55 (4000V, 3 hr). The loading 170 μg.

al SVPD digestion yielded fragments ($m^1$ApUp, Cp)C, ($m^1$ApUp, Cp, Cp)U and ($m^1$ApUp, Cp, Cp, Up)C. These data (see also Table 1) gave the sequence of t13- $m^1$ApUpCpCpUpCpGp.

Oligonucleotide t14. Exonuclease A-5 digestion yielded two products CpD and (Cp, Dp)A. One of them didn't show any

Table 1. Guanylo-RNase digestion products of $tRNA_{2b}^{Val}$
(the oligonucleotide number refers to Fig.2)

| Oligonucleotide number | Composition | Pancreatic RNase digestion in the presence of alkaline phosphatase | Sequence | Molar yield[e] |
|---|---|---|---|---|
| t1 | G | | Gp | 5.5 |
| t2 | C:A=0.94:1.0 | AACp:ACp:C=0.8:0.96:1.0[a] | AACACCcyH | 0.85 |
| t3 | C:G=1.0:1.0 | | CGp | 1.4 |
| t4 | A:G=0.83:1.0 | | AGp | 1.3 |
| t5 | U:G=0.8:1.0 | | UGp | 1.3 |
| t6 | G | | pGp | 1.2 |
| t7 | Ψ:A:G=0.96:0.87:1.0 | Ψ:AG=0.8:1.0 | ΨAGp | 0.95 |
| t8 | C:A:G=1.03:3.0:1.0 | C:AAAG=1.2:1.0(A:G=3.1:1.0) | CAAAGp | 1.2 |
| t9 | $m^7G$:C:$m^5C$:G=0.96:1.8:0.9=1.0 | $m^7GD$:C:$m^5C$:G=0.9:2.14:0.94:1.0 | $m^7GDCm^5CCGp$ | 1.0 |
| t10 | U:G=1.62:1.0 | | UUGp | 0.77 |
| t11 | Ψ:G=1.7:1.0 | | ΨΨGp | 0.82 |
| t12 | T:Ψ:C:G=1.12:0.9:0.8:1.0 | | TΨCGp | 0.73 |
| t13 | $m^1A$:U:C:G=0.9:1.7:3.1:1.0 | $m^1AU$:C:U:G=0.8:3.3:0.94:1.0 | $m^1AUCCUCGp$ | 0.83 |
| t14 | C:A:U:$m^2G$=3.2:2.18:0.7:1.0[b] | AC:AU:C:$m^2G$=0.9:0.88:1.8:1.0 | $CDAUCACm^2Gp$ | 0.94 |
| t15 | C:Ψ:U:A:G=4.7:0.73:0.68:2.1:1.0 | AC:CΨ:U:G=2.0:2.9:0.65:0.7:1.0 | CCΨUCACACGp | 0.78 |
| t16 | U:C:A:G=2.7:2.1:3.1:1.0[c] | AAU:C:U:AG=1.1:1.9:1.7:1.0[d] | UUCCAAUAGp | 0.96 |

a) digestion without phosphatase
b) during the analysis in nucleotide form Dp was found (0.75 mole)
c) mixture $m^2G$ and G (0.43:1.0); d)mixture $Am^2G$ and AG (0.4:1.0)
e) mole per mole $m^7GDC^5CCGp$

nucleoside after $T_2$ RNase treatment, that suggested the presence of D in this position; Dp was identified in the latter from the alkaline spectra as described in section "Materials and Methods". Moreover the complete SVPD digestion indicated C as 5'-end of t14. Among the products of the partial pancreatic RNase digestion fragments (Ap, Cp, $m^2Gp$) and (Cp, Dp, Ap, Up, Cp) were found. These results in connection with other data (Table 1) gave the sequence $CpDpApUpCpApCpm^2Gp$ for t14.

Oligonucleotide 15. The partial digestion with exonuclease A-5 yielded (Cp, Cp)Ψ and (Cp, Cp, Ψp)U. Two fragments (Ap, Cp, Ap, Cp, Gp) and (Ap, Cp, Gp) were obtained after the partial digestion of t15 with pancreatic RNase. These data together with the results of the complete pancreatic RNase digestion led to the sequence CpCpΨpUpCpApCpApCpGp.

Oligonucleotide t16. 3'-end of t16 consisted of 70% Gp and 30% $m^2Gp$. Exonuclease A-5 digestion yielded UpU and UpUpC. Since among the products of endonuclease A-236 digestion pCpC, pApU and pApA were found, this information plus data of the pancreatic RNase digestion gave the sequence of t16 as UpUpCpCpApApUpApGp.

The results of the sequence analysis are listed in Table 1.

Pancreatic RNase digest. The products of the pancreatic RNase digestion were separated as described above; fingerprint is shown in Fig.3. The results of the analysis of oligonucleotides and their molar yield are summarized in Table 2.

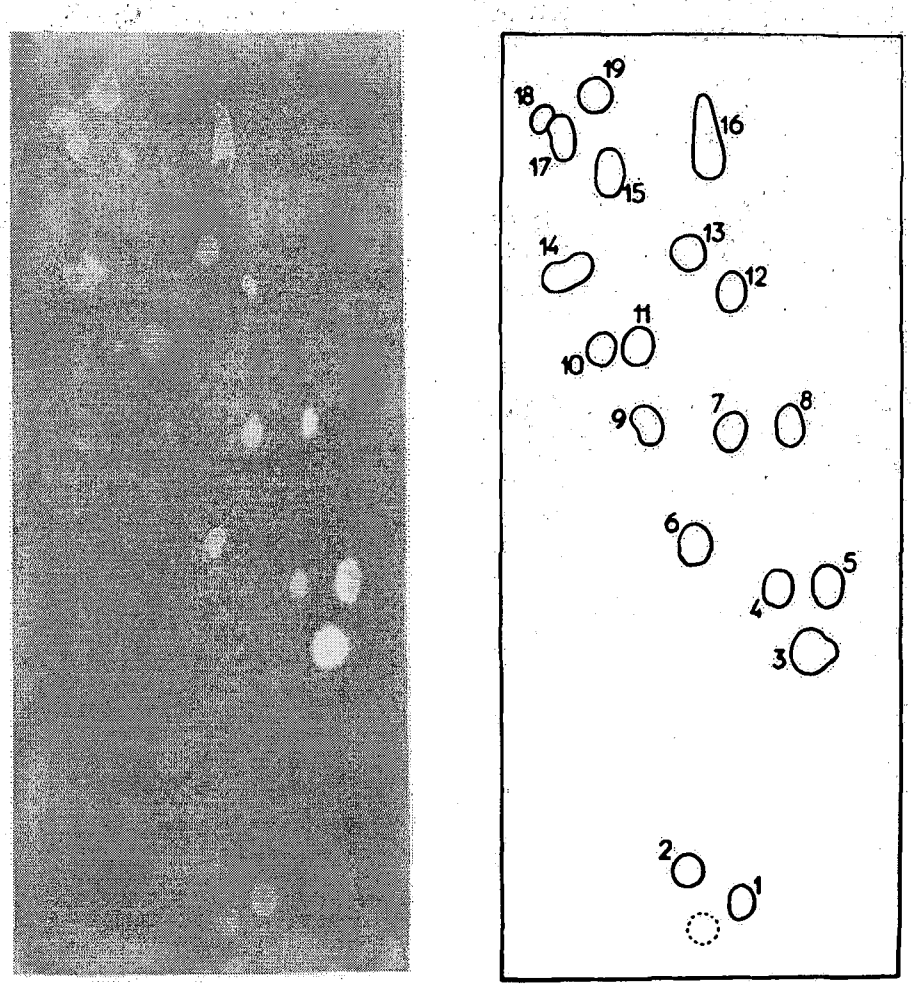



Fig.3. Fingerprint of a complete pancreatic RNase digest of baker's yeast $tRNA^{Val}_{2b}$. First dimension, left to right, TLC on cellulose in system B. Second dimension, top to bottom, electrophoresis on DEAE-paper at pH=1.7 (4000V, 2 hr). The loading 270 μg.

Table 2. Pancreatic RNase digestion products of $tRNA^{Val}_{2b}$
(the oligonucleotide number refers to Fig.3)

| Oligonucleotide number | Composition | Guanylo-RNase digestion in the presence of alkaline phosphatase | Sequence | Molar yield (mole per mole AAAGm$^7$GDp) |
|---|---|---|---|---|
| p1 | U | | Up | 4.5 |
| | Dp[a] | | Dp | 0.7 |
| p2 | Ψ | | ΨP | 2.5 |
| p3 | C | | Cp | 15.2 |
| | m$^5$C | | m$^5$Cp | 1.1 |
| p4 | A:U=1.2:1.0 | | AUp | 1.2 |
| p5 | A:C=0.8:1.0 | | ACp | 4.4 |
| p6 | G:C=1.3:1.0 | | GCp | 1.4 |
| p7 | A:G:C=1.0:0.7:1.0 | AG:C=1.3:1.0 | AGCp | 1.3 |
| p8 | A:U=2.1:1.0 | | AAUp | 1.2 |
| p9 | m$^2$G:Ψ=1.26:1.0 | | m$^2$GΨp | 0.72 |
| p10 | G:Ψ=1.1:1.0 | | GΨp | 0.97 |
| p11 | G:m$^1$A:U=1.2:1.05:1.0 | G:m$^1$AU=1.18:1.0 | Gm$^1$AUp | 0.9 |
| p12 | A:m$^2$G:U=1.2:1.14:1.0 | Am$^2$G:U=1.04:1.0 | Am$^2$GUp | 0.4 |
| p13 | A:G:U=1.2:1.2:1.0 | AG:U=1.25:1.0 | AGUp | 0.75 |
| p14 | G:C=2.05:1.0 | | GGCp | 1.4 |
| p15 | G:A:C=1.88:2.15:1.0 | G:AAC=1.75:1.0 | GGAACp | 0.6 |
| p16 | A:G:m$^7$G=3.3:1.1:1.0 | AAAG:m$^7$GD=1.2:1.0 | AAAGm$^7$GDp | 1.0 |
| p17 | G:U=2.1:1.0 | | GGUp | 0.7 |
| | pGp:Up=0.9:1.0[a] | | pGUp | 0.7 |
| p18 | G:C=2.5:1.0 | | GGGCp | 0.4 |
| p19 | G:A:T=2.2:1.15:1.0 | | GAGTp | 0.78 |

a) analysis in nucleotide form (systems B and E)

Dp was found in the spot corresponding to Up after the measuring of alkaline spectra. Cp and m$^5$Cp were resolved in the dephosphorylated form by TLC in systems A and B. Oligonucleotides p12 (Apm$^2$GpUp) and p13 (ApGpUp) represent the various sequences of the same position of $tRNA^{Val}_{2b}$. Oligonucleotides GpGpUp and pGpUp (p17) were separated by TLC in system E. Oligonucleotide p18 (GpGpGpCp) was present in amount of 0.4 mole and wasn't the component of $tRNA^{Val}_{2b}$ (as became clear during the analysis of the halves of $tRNA^{Val}_{2b}$, see below). The only oligonucleotide, which couldn't be identified by the combined results of $T_2$ RNase and guanylo-RNase digestion, was oligonucleotide p19. Its sequence – GpApGpTp– was established after exonuclease A-5 digestion gave dinucleosidemonophosphate GpA.

The obtaining and analysis of the halves of $tRNA^{Val}_{2b}$. The cleavage of $tRNA^{Val}_{2b}$ into two halves with pancreatic RNase was

performed by modified method of Penswick and Holley [27]. The modification was based on the observation that the degree of hydrolysis of the intact tRNA at 0°C in presence of $Mg^{2+}$ depends largely on the concentration of $Mg^{2+}$ [28]. Except halves no products were formed in 100 mM solution of magnesium acetate (Fig.4). The separated on DEAE-cellulose and desalted halves were completely digested with guanylo-RNase and pancreatic RNase and the products were fractionated the same way as the digests of the intact molecule (Fig. 5, 6).

In the guanylo-RNase digests of the halves, as be expected, there were several new spots, which were absent on the fingerprint of the intact $tRNA^{Val}_{2b}$. Oligonucleotide CpCpΨpUpCpApCp-ApCpGp vanished and instead of it in 3'-half appeared ApCpGp and in 5'-half appeared CpCpΨpUpCp. Besides oligonucleotide $ApApCpApCpC_{OH}$ from 3'-half changed its position on fingerprint and was identified as ApApCpApCp. Obviously these alterations were caused by the action of pancreatic RNase during the cleavage of $tRNA^{Val}_{2b}$ into two halves.

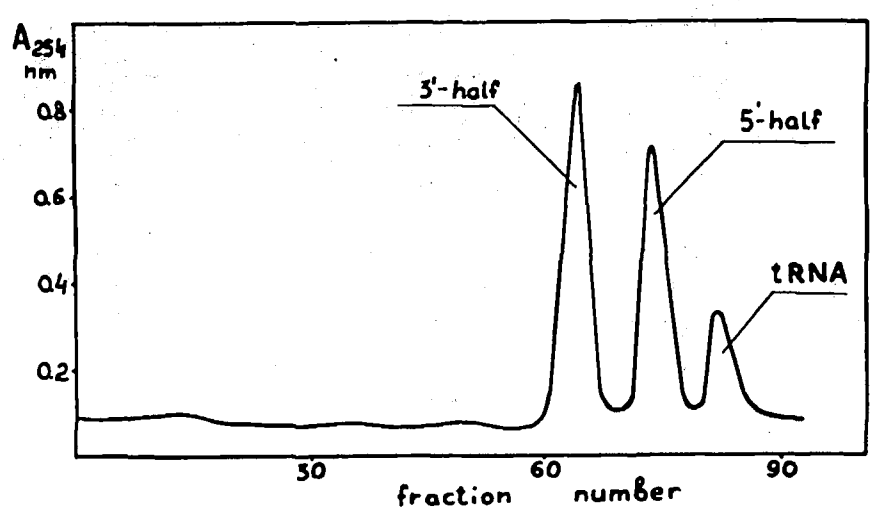



Fig.4. The separation of the partial pancreatic RNase digestion products of $tRNA^{Val}_{2b}$ on DEAE-cellulose in 7M urea, pH=3.4 with a linear gradient of NaCl (0 to 0.6 M). Column 6.6 ml (h=40 cm), elution volume 200 ml, flow rate 2.6 ml/hour. The loading 460 μg.

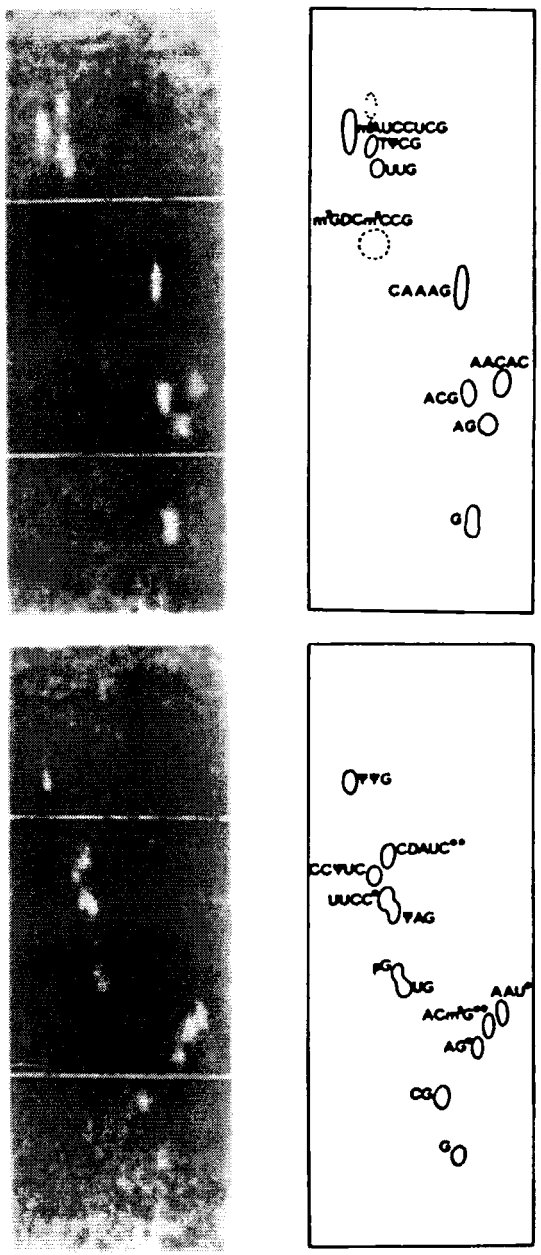



Fig.5. Fingerprints of complete guanylo-RNase digests of the halves of baker's yeast $tRNA^{Val}_{2b}$. Separation conditions see in Fig.2. The loading 70 µg. a) 3'-half; b) 5'-half.

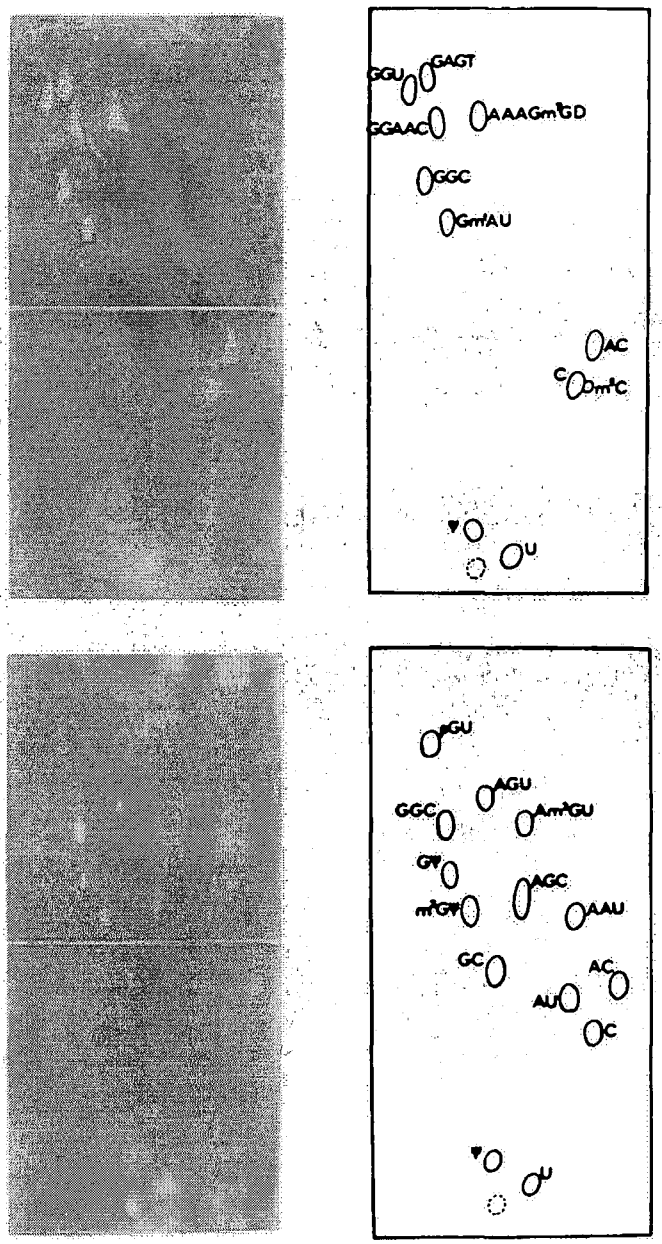



Fig.6. Fingerprints of complete pancreatic RNase digests of the halves of baker's yeast tRNA$^{Val}_{2b}$. Separation conditions see in Fig.3. The loading 70 µg. a) 3'-half; b) 5'-half.

In 5'-half also unspecific degradation took place: oligonucleotide UpUpCpCpApApUpApGp was destructed into 3 fragments UpUpCpCp, ApApUp and ApGp; oligonucleotide CpDpApUpCpApCpm$^2$Gp was destroyed into 2 fragments CpDpApUpCp and ApCpm$^2$Gp. It made difficult the identification of the spots but on the other hand confirmed indirectly the sequence of these oligonucleotides. The fragments of the halves were identified (except of the above cases) from their mobility $R_{Gp}$ for guanylo-RNase digestion and $R_{Up}$ for pancreatic RNase digestion.

The analysis of the halves allowed to correct some molar yields of the components (see Tables 3, 4)It was important that trinucleotide GpGpCp was present in the both halves and tetranucleotide GpGpGpCp completely absent. This fact led to the conclusion that there are two oligonucleotides GpGpCp in $tRNA^{Val}_{2b}$ and GpGpGpCp observed in the digest of the intact molecule doesn't belong to $tRNA^{Val}_{2b}$. Its presence was a result of the preparation of $tRNA^{Val}_{2b}$ to be not 100% pure; on the fingerprints there were other contaminating spots, but their amounts were uncomparable with that of the major oligonucleotides. Such a high content of GpGpGpCp can be partly explained by the fact that it was poorly separated from pGpUp and

Table 3. Guanylo-RNase digestion products of the halves of $tRNA^{Val}_{2b}$

3'-half

| Oligonucleotide | Molar yield (mole per mole m$^7$GDCm$^5$CCGp) |
|---|---|
| Gp | 2.15 |
| AGp | 1.25 |
| ACGp | 1.2 |
| AACACp | 0.8 |
| CAAAGp | 1.0 |
| m$^7$GDCm$^5$CCGp | 1.0 |
| UUGp | 0.97 |
| m$^1$AUCCUCGp | 0.84 |
| TΨCGp | 1.05 |

5'-half

| Oligonucleotide | Molar yield (mole per mole ΨAGp) |
|---|---|
| Gp | 2.2 |
| CGp | 1.3 |
| AAUp* | 1.06 |
| ACm$^2$Gp** | 1.06 |
| UGp | 1.2 |
| pGp | 1.2 |
| ΨAGp | 1.0 |
| UUCCp* | 0.8 |
| CCΨUCp | 0.7 |
| CDAUCp** | 1.05 |
| ΨΨGp | 1.15 |
| AGp* | 0.8 |

Asterisks refer to Fig.6

Table 4. Pancreatic RNase digestion products of the halves of $tRNA^{Val}_{2b}$

3'-half

| Oligonucleotide | Molar yield (mole per mole AAAGm$^7$GDp) |
|---|---|
| ACp | 2.07 |
| GGUp | 0.9 |
| GAGTp | 1.1 |
| AAAGm$^7$GDp | 1.0 |
| GGAACp | 0.95 |
| GGCp | 1.1 |
| Gm$^1$AUp | 0.85 |
| Up | 1.9 |
| ΨΨp | 1.05 |
| Cp | 5.8 |
| m$^5$Cp | 0.8 |

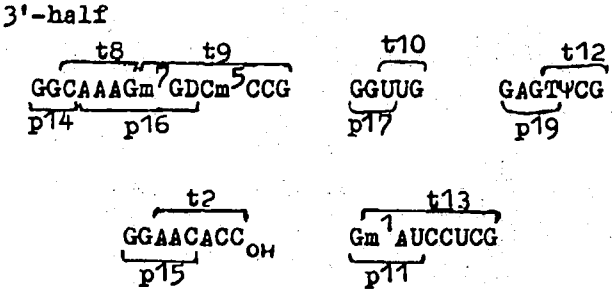
5'-half

| Oligonucleotide | Molar yield (mole per mole GΨp) |
|---|---|
| AAUp | 0.95 |
| ACp | 0.95 |
| GCp | 0.78 |
| AGCp | 1.03 |
| m$^2$GΨp | 1.0 |
| GΨp | 1.0 |
| AUp | 0.94 |
| pGUp | 0.83 |
| AGUp | 0.76 |
| Am$^2$GUp | 0.3 |
| GGCp | 1.0 |
| Up | 1.9 |
| ΨΨp | 1.8 |
| Cp | 4.75 |

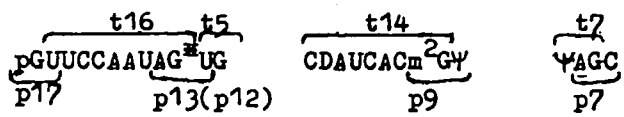
GpGpUp and contained a material of these oligonucleotides (see Fig.3).

Reconstruction of the molecule $tRNA^{Val}_{2b}$. The analysis of the halves enabled to distribute the oligonucleotides in two parts of the molecule and the comparison of the complete digests gave the overlapping sequences (G* - mixture G and m$^2$G):

3'-half




5'-half




However this information is not sufficient to reconstruct $tRNA^{Val}_{2b}$. Comparing all determined up to now primary structures of tRNAs it can be seen that inspite of their variety some positions in them are occupied by the same nucleotide

(the most modern and complete review of these data was made by Dirheimer et al. [29]). For example, if tRNA has ribothymidine, it always locates in 23 position from 3'-end of molecule. The other common positions are $A_{14}$ (numeration from 5'-end) and tandem GG in the dihydrouridilic loop. Thus fixing the position of the fragment containing T and taking in account two end fragments of 3'-half one can unambiguously reconstruct this part of the molecule:

ACG GAGTΨCG GGUUG

GGCAAAGm$^7$GDCm$^5$CCG Gm$^1$AUCCUCG GGAACACC$_{OH}$

Similarly the fixation of $A_{14}$ enables to write the tetramer ΨAGCp immediately after the 5'-end fragment pGUUCCAAUAG$^{*}$UGp of 5'-half of the molecule; it gives an overlapping sequence

t14 t11

CDAUCACm$^2$GΨΨG

p9

The location of GG-tandem into the middle of 5'-half, in D-loop, leads now to the complete reconstruction of this half of tRNA$^{Val}_{2b}$:

pGUUCCAAUAG$^{*}$UGΨAGC CDAUCACm$^2$GΨΨG

GGC CCΨUC

The assembled structure of tRNA$^{Val}_{2b}$ can be arranged in the cloverleaf model (Fig.7). There are 7 base-pairs in its ac-

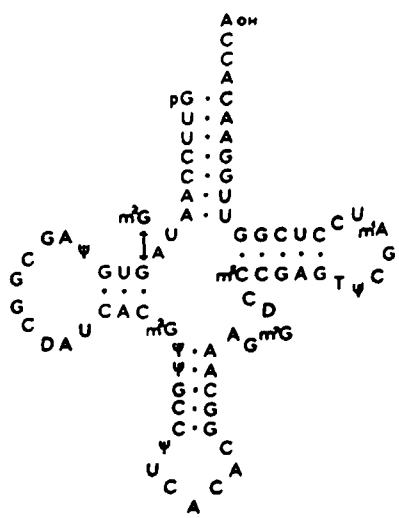


Fig.7. The primary structure of baker's yeast tRNA$^{Val}_{2b}$ in the cloverleaf form.

ceptor stem, 5 base-pairs in anticodon and TΨ-stem and 3 base-pairs in D-stem.

DISCUSSION

A small amount of $tRNA_{2b}^{Val}$ available for this work made some difficulties in the analysis of the primary structure using UV-absorbing methods. The determination of the nucleotide sequence of $tRNA_{2b}^{Val}$ was carried out with 3 mg tRNA. It was made possible by using TLC on cellulose for the studying of the composition and sequence of the oligonucleotides. Often the components were identified from their chromatographic mobilities comparing with the mobilities of markers because of lower sensitivity of spectral method. The fingerprinting system developped in our laboratory [16] allows to separate practically all fragments of guanylo-RNase and pancreatic RNase digests of tRNA. Moreover the electrophoretic mobility of oligonucleotides happened to be higher at pH=1.5-1.55 than at pH=1.7. This pH together with a low and constant temperature of the cooling plate (+2° - +4°C) prevents the diffusion of spots and improves the resolution of components and reproducability of the electrophoresis. The high reproducability of the system allowed to identify oligonucleotides of the halves of $tRNA_{2b}^{Val}$ from their mobility.

Capacity of one fingerprint is about 300 μg of digest of tRNA therefore necessary amount of material was obtained from 4 fingerprints (for guanylo-RNase digest) and from 2 fingerprints (for pancreatic RNase digest).

The amount of $tRNA_{2b}^{Val}$ was sufficient only for analysis of the complete RNase digests of tRNA and its halves. The selection of the conditions for producing of fragments shorter than halves of tRNA (metamers) might require more material than the whole preceding investigation. On the other hand taking into consideration three common for all tRNAs positions - location of $A_{14}$ and tandem GG in 5'-half and ribothymidine in 3'-half one can assemble the primary structure of $tRNA_{2b}^{Val}$. The same nucleotide sequence can be obtained basing on the cloverleaf model and principle of the maximum base-pairing. The coincidence of the two reconstructions obtained

from two independent assumptions make any other primary structure of $tRNA_{2b}^{Val}$ so unlikely that we decided to spend no time and efforts for preparing the additional amounts of $tRNA_{2b}^{Val}$, metamers from it and their analysis by UV-absorbing methods.

The molecule of baker's yeast $tRNA_{2b}^{Val}$ is shorter by one residue than $tRNA_{1}^{Val}$ and $tRNA_{2a}^{Val}$ from the same source [2,30]; it consists of 76 nucleotides. The sequence of $tRNA_{2b}^{Val}$ differs from $tRNA_{1}^{Val}$ in 26 points, besides $D_{16}$ is quite absent in the former. From $tRNA_{2a}^{Val}$ it differs in 15 points, $C_{21}$ is quite absent in $tRNA_{2b}^{Val}$ (numbers $D_{16}$ and $C_{21}$ refer to positions of these nucleosides in $tRNA_{1}^{Val}$ and $tRNA_{2a}^{Val}$, respectively). Moreover 30% of molecules $tRNA_{2b}^{Val}$ have $N^2$-methyl-guanosine in position 10 (as $tRNA_{2a}^{Val}$) and 70% have guanosine (as $tRNA_{1}^{Val}$).

The interesting feature of $tRNA_{2b}^{Val}$ is the presence of the second $m^2G$ between the anticodon and dihydrouridilic loops, where $m_2^2G$ usually occurs. It is the first case for baker's yeast tRNAs. Only in two mammalian tRNA species -valine tRNA from mouse myeloma cells [31] and from rabbit liver [32] and initiator tRNA from several sources [33-35] this position is occupied by $m^2G$.

This point is not the single one relating baker's yeast $tRNA_{2b}^{Val}$ with mammalian valine tRNA (valine tRNAs from mouse myeloma and rabbit liver are identical). There are the whole nucleotide sequences in 6-8 residues common for these tRNAs; the length of polynucleotide chain is the same too. Differences between these tRNAs are even less than between $tRNA_{1}^{Val}$ and $tRNA_{2b}^{Val}$ from baker's yeast. Generally yeast valine tRNAs resemble mouse myeloma valine tRNA much more than valine tRNAs from bacteria [36-38]. It may indicate a certain conservation of tRNAs during the evolution of eukaryotes and the essential distinction between eukaryotes and prokaryotes in structural arrangement of even such an ancient molecular form as tRNA.

The high structural dissimilarity of baker's yeast valine tRNAs may implies their different function and different intracellular localization. The data obtained by Martin, et al. [39] showed the great difference between cytoplasmic and mitochondrial tRNAs from yeast. There were not found $m^1A$,

$m^5C$ and $m^7G$ in mitochondrial tRNA and it has a low G+C content - 35%. According to these criteria all three baker's yeast valine tRNAs are not mitochondrial: they have $m^1A$ and $m^5C$ and two of them - $m^7G$; their G+C content is similar to cytoplasmic tRNA. It must be noticed that the investigation of Martin, et al. was made on the crude yeast tRNA and doesn't reflex special features of particular tRNAs.

$tRNA^{Val}_{2b}$ is the first sequenced valine tRNA having cytidine in the first position of anticodon. The only codon which can correspond to it is GUG, one of the initiator triplets, therefore $tRNA^{Val}_{2b}$ possibly can initiate the protein synthesis.

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