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INFLUENCE OF MODIFIED NUCLEOSIDES IN *E. coli* TRANSFER RIBONUCLEIC ACIDS ON CHROMATOGRAPHIC MOBILITIES OF TRANSFER RNA

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SUMMARY

The relationship between structure and partition coefficient K of 23 *E. coli* tRNA species has been investigated. Fractionation was performed by counter-current distribution in two salt–solvent systems containing phosphate buffer and two organic components: 2-methoxy ethanol and 2-butoxy ethanol (PMB system), formamide and isopropanol (PFI system). For the tRNAs studied, dependence of K on nucleoside composition is described by the relationship: $\log K = c(\mathrm{A/Y}) + d$. The values for d vary over an interval Δd, which is a function of the polarity of the anticodon loop. The modified nucleosides are contained mainly in this highly exposed region of the tRNA molecule.

The tRNAs fall into three groups according to the anticodon loop polarity: group 1 with the lowest d value (hydrophilic anticodon loop) includes $\mathrm{tRNA}^{\mathrm{Ala}}_{1\mathrm{A}}$, $\mathrm{tRNA}^{\mathrm{Ile}}$, $\mathrm{tRNA}^{\mathrm{Lys}}$, $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{M}}$, $\mathrm{tRNA}^{\mathrm{Ser}}_{1,3}$, $\mathrm{tRNA}^{\mathrm{Thr}}$ and $\mathrm{tRNA}^{\mathrm{Val}}_{1}$, each containing a polar N-(purin-6-yl carbamoyl)-threonine riboside, or a 5-oxyacetic uridine acid in the anticodon loop; group 2 with neutral anticodon loop containing 2-methyl adenosine and/or a modified 2-thio uridine is composed with $\mathrm{tRNA}^{\mathrm{Arg}}_{1,2}$, $\mathrm{tRNA}^{\mathrm{Asp}}_{1}$, $\mathrm{tRNA}^{\mathrm{GlX}}_{1,2}$, $\mathrm{tRNA}^{\mathrm{Gly}}_{1\text{-}3}$, $\mathrm{tRNA}^{\mathrm{His}}_{1}$, $\mathrm{tRNA}^{\mathrm{Leu}}_{1,2}$, $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{F}}$ and $\mathrm{tRNA}^{\mathrm{Val}}_{2\mathrm{A}}$; group 3 with the highest d value (lipophilic anticodon loop) comprises $\mathrm{tRNA}^{\mathrm{Phe}}$, $\mathrm{tRNA}^{\mathrm{Trp}}$ and $\mathrm{tRNA}^{\mathrm{Tyr}}$, characterized by a lipophilic 2-methylthio N^6-isopentenyl adenosine at the 3′-end of the anticodon. For similar overall composition, the order of increasing mobility of tRNAs leads to an increase in Δd, which depends on the decreasing polarity of the modified nucleosides located in the anticodon loop.

INTRODUCTION

Analytical or preparative methods used in the fractionation of tRNA species

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by liquid–liquid continuous separation (partition chromatography) and discontinuous separation (counter-current distribution, CCD) are based on different partition coefficients (K) for different tRNA species. The relation between partition coefficient and the tRNA structure has been studied by Garel and Mandel[1] for yeast tRNAs. Within a homogenous family of polynucleotides like the tRNA species (80 $\pm$ 5 nucleotides, helix content 54 $\pm$ 3%), the partition coefficient is mainly a function of the total nucleotide composition of the tRNA, expressed by the relationship $\log K = A/Y$, where A and Y represent the number of adenosine and pyrimidine nucleoside residues, respectiely. This relation has been checked for oligoribonucleotides[2] and fragments of rRNA[3].

For prokaryotic tRNA species, one expects a comparable mobility relationship. Nevertheless, it must be noted that modified nucleosides are less numerous and mainly located in the anticodon loop. Several experimental approaches —associations with codons and oligonucleotides[4], chemical reactivity[5–7], enzymic degradability[8]— have shown that this loop is exposed. The tertiary structure of $tRNA^{Phe}$ confirms the accessibility of the anticodon loop (see reviews[9,10]). We have therefore considered the influence of the degree of polarity of the anticodon loop on the chromatographic mobility of tRNA species fractionated by CCD.

MATERIALS AND METHODS

tRNA and L-amino acid:tRNA ligases (EC 6.1.1.)

E. coli B tRNA was purchased from Calbiochem (San Diego, Calif., U.S.A.). [^{14}C]amino acids used for their *in vitro* acylation assays were provided by CEA (Saclay, France). tRNA ligases were prepared as described by Chavancy *et al.*[11] from a post-ribosomal supernatant of *E. coli* culture generously given by G. Dretzen (Strasbourg, France) and used in standard conditions (Mg^{++}/ATP = 1.5).

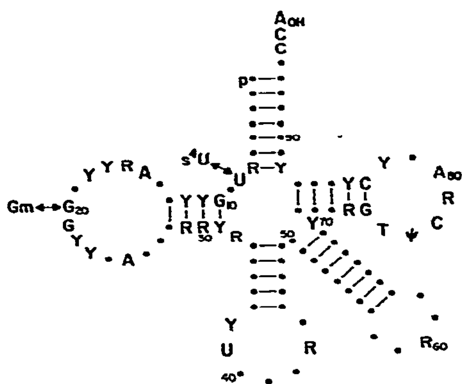
Counter-current distribution

160 Transfers were made with 600 A_{260} units of *E. coli* B tRNA at 15° in the PMB solvent system containing 18.6% 2-butoxy ethanol as previously described[1] (1,200 ml of 1.50 *M* potassium phosphate buffer (pH 7.0), 400 ml 2-methoxy ethanol, 365 ml 2-butoxy ethanol and 1.6 ml 1 *M* $MgCl_2$). In these conditions the average partition coefficient is about 1. Extraction of tRNA fractions, acylations and calculations of the partition coefficient of specific isoaccepting tRNA species have been described[12]. Statistical studies of the base distribution in the whole molecule of tRNA and the distribution of purine bases in the anticodon region (stem and loop) were carried out by J. L. Chasse (Laboratoire de Biométrie, Université Claude Bernard, Lyon I, France) with a Wang 700 calculator.

RESULTS

Structural data

The 28 tRNA sequences of *E. coli* B and K count 37 homologous positions for 90% of the molecules studied (Fig. 1). The most marked structural modification is found in the arms with paired bases and in the three bases of the anticodon. The numbering used (1–98) is based on the principle of maximum recovery of primary



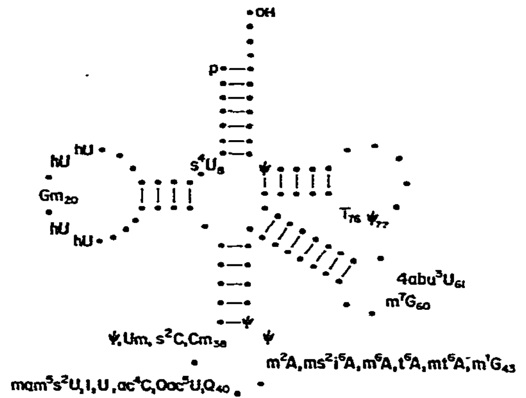


Fig. 1. Common nucleotides in *E. coli* tRNA species. The numbering used (1–98) is based on the principle of maximum overlapping of the primary structures of 28 *E. coli* tRNAs. The letters indicate the nature and position of nucleotides common to more than 90% of the tRNAs studied. A = adenosine-5′-phosphate; C = cytidine-5′-phosphate; G = guanosine-5′-phosphate; U = uridine-5′-phosphate; R = purine riboside-5′-phosphate; Y = pyrimidine riboside-5′-phosphate; T = ribothymidine-5′-phosphate; ψ = pseudouridine-5′-phosphate. For modified nucleotides, see legend of Table II.

Fig. 2. Modified nucleosides in *E. coli* tRNA species. The numbering is as in Fig. 1. m^1G = 1-methyl guanosine; m^2G = 2-methyl guanosine. For other abbreviations see legend of Table II.

TABLE I

STRUCTURAL DATA FOR 28 *E. COLI* tRNA SPECIES

tRNA	*A*	*G*	*C*	*U + hU*	ψ	*T*	*N*	*Total*	*R/Y*	*A/A + G (%)*	*A/Y (%)*	*Refs.*
Ala_{1A}	11	27	24	11	1	1		75	1.03	29.0	29.8	13
Arg_1	15	23	23	12	1	1	I	76	1.05	39.5	40.5	14, 15, 16
Arg_2	16	23	23	12	1	1	I	77	1.02	41.0	41.8	17
Asp_1	11	27	22	14	2	1		77	0.97	29 0	28.2	18
Gln_1	15	21	22	14	2	1		75	0.92	41.6	38.4	19, 20
Gln_2	15	21	23	12	3	1		75	0.92	41.6	38.4	19
Glu_1	13	23	27	10	2	1		76	0.90	36.1	32.5	21
Glu_2	14	22	27	10	2	1		76	0.90	39.0	35.0	22
Gly_1	15	20	22	15	1	1		74	0.90	42.8	38.5	23
Gly_2	13	19	24	17	1	1		75	0.74	40.6	30.2	24, 25
Gly_3	13	25	21	15	1	1		76	1.00	34.2	34.2	26
His	14	22	19	18	3	1		77	0.88	38.9	34.2	27, 28
Ile_1	16	25	19	14	2	1		77	1.14	39.0	44.5	29, 16
Leu_1	15	29	24	15	3	1		87	1.02	34.1	34.9	30, 31
Leu_2	17	29 ·	21	17	2	1		87	1.12	37.0	41.5	30, 31
Lys	17	20	18	17	2	1	X	76	0.95	46.0	43.6	32
$Met^F_{1,2}$	15	24	26	10	1	1		77	1.03	38.5	39.5	33, 34
Met^M	19	20	20	15	2	1		77	1.02	48.7	50.0	35, 36
Phe	15	24	21	12	3	1		76	1.05	38.5	40.5	37, 16
Ser_1	19	29	24	14	1	1		88	1.20	39.6	47.5	38, 39
Ser_3	18	32	28	13	1	1		93	1.16	36.0	41.9	40, 41
Thr	17	23	18	16	1	1		76	1.11	42.5	47.0	42
Trp_{su}	15	23	21	15	1	1		76	1.00	39.5	39.5	43
$Tyr_{1,su}$	19	23	27	13	2	1		85	0.98	45.2	44.1	44
Tyr_2	20	25	27	12	2	1		87	1.07	44.4	47.6	45
Val_1	15	24	23	12	1	1		76	1.05	38.5	40.5	46, 47, 48
Val_{2A}	13	26	21	15	1	1		77	1.02	33.3	34.2	49, 16
Val_{2B}	16	23	18	18	1	1		77	1.02	41.0	42.0	49, 16

structures. The chain length varies mostly in the extra arm (positions 50–70), whereas it is constant for the dihydrouridine region (positions 10–31).

Table I lists some structural data. The molar ratio of purines to pyrimidines (R/Y) changes from 0.88 for $tRNA_1^{His}$ to 1.20 for $tRNA_1^{Ser}$ while the relative proportion of A to G, expressed by the ratio A/(A + G), varies from 0.29 for $tRNA_1^{Asp}$ or $tRNA_{1A}^{Ala}$ to 0.487 for $tRNA_M^{Met}$. The chain lengths are very close for 22 tRNA species (76 ± 1 nucleotides) and higher for 6 species: $tRNA^{Leu}$, $tRNA^{Ser}$ and $tRNA^{Tyr}$ (89 ± 4 nucleotides).

Fig. 2 shows the positions generally occupied by modified nucleosides and Table II indicates the nature and location of these modified nucleosides clustered mainly in the anticodon loop.

TABLE II

ALKYLATED BASES AND MODIFIED NUCLEOSIDES IN *E. COLI* tRNA SPECIES

tRNAs are arranged according to the three groups shown in the semi-logarithmic plots based upon the polarity of the anticodon loop (Figs. 6 and 7). U* = unknown modification; $4abu^3U$ = 3-(3-amino 3-carboxypropyl) uridine[15,16]; Q = 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-yl aminomethyl) 7-deazaguanosine[57]; m^2A = 2-methyl adenosine; m^6A = N^6-methyl adenosine; ms^2i^6A = 2-methylthio N^6-isopentenyl adenosine; t^6A = N-(purine-6-ylcarbamoyl)threonine riboside; mt^6A = N-methyl N-(purine-6-ylcarbamoyl) threonine riboside; ac^4C = N^4-acetyl cytidine; s^2C = 2-thio cytidine; Cm = 2′-O-methyl cytidine; m^7G = N^7-methyl guanosine; Gm = 2′-O-methyl guanosine; I = inosine; oac^5U = 5-oxyacetic uridine acid; s^4U = 4-thio uridine; mam^5s^2U = 5-methylaminomethyl 2-thio uridine; hU = 5,6-dihydrouridine; Um = 2′-O-methyl uridine; ψ = pseudouridine.

tRNA	*Position 8*	*hU loop*	*Anticodon loop*			*Extra arm*
			Position 38	*Wobble base, position 40*	*Position 43*	
Ala_{1A}	s^4U			oac^5U		m^7G
Ile					t^6A	m^7G, $4abu^3U$
Lys				mam^5s^2U	t^6A	m^7G, X
Met^M	s^4U	Gm		ac^4C	t^6A	m^7G
Ser_1	s^4U	m^2G	Cm	oac^5U	ms^2i^6A	
Ser_3	s^4U		s^2C		t^6A	
Thr					mt^6A	
Val_1	s^4U			oac^5U	m^6A	m^7G
Arg_1	s^4U		s^2C	I	m^2A	m^7G, $4abu^3U$
Arg_2	s^4U			I	m^2A	m^7G, $4abu^3U$
Asp_1	s^4U			Q	m^2A	m^7G
Gln_1	s^4U	Gm	Um	mam^5s^2U	m^2A	
Gln_2	s^4U	Gm	Um		m^2A	
Glu_1				mam^5s^2U	m^2A	
Glu_2	s^4U			mam^5s^2U	m^2A	
Gly_1	s^4U					
Gly_2				U*		
Gly_3						m^7G
His_1	$(s^4U)_9$			Q	m^2A	m^7G
$Leu_{1,2}$		Gm			m^1G	
Met^F	s^4U		Cm			
Val_{2A}	s^4U					m^7G, $4abu^3U$
Phe	s^4U		ψ		ms^2i^6A	m^7G, $4abu^3U$
Trp	s^4U		Cm		ms^2i^6A	m^7G
$Tyr_{1,2}$	s^4U	Gm		Q	ms^2i^6A	

TABLE III

OBSERVED AND THEORETICAL DISTRIBUTION OF IDENTICAL BASE SEQUENCES FOR *E. COLI* tRNA SPECIES

The upper lines give the observed distribution, the lower lines that theoretical distribution[1]. n_A is the number of nucleosides A in the tRNA, n_G the number of nucleosides G, I, etc.; r is the number of consecutive sequences in the tRNA; for instance, r_3 indicates the frequency of NNN in the sequence, N is successively A, G, C, U (including ψ and T); for $r_{>3}$, the number of consecutive sequences is shown in parentheses after the figure giving the observed distribution.

tRNA	*A*					*G, I*				
	n_A	r_1	r_2	r_3	$r_{>3}$	n_G	r_1	r_2	r_3	$r_{>3}$
Ala_{1A}	10	10	0	0	0	26	13	4	0	1 (5)
		7.61	0.99	0.12	0.01		10.83	3.92	1.38	0.71
Arg_1	14	10	2	0	0	24	12	6	0	0
		9.37	1.74	0.30	0.06		11.04	3.63	1.16	0.51
Asp_1	10	8	1	0	0	27	13	3	1	1 (5)
		7.59	0.95	0.11	0.01		11.05	3.99	1.40	0.72
Gln_1	14	8	3	0	0	21	5	6	0	1 (4)
		9.37	1.74	0.30	0.06		10.89	3.11	0.86	0.28
Glu_2	13	9	2	0	0	22	6	4	0	2 (4)
		9.05	1.53	0.24	0.04		11.10	3.28	0.94	0.35
Gly_3	12	8	2	0	0	25	14	2	1	1 (4)
		9.00	1.51	0.21	0.04		11.19	3.78	1.24	0.57
His_1	13	11	1	0	0	22	11	4	1	0
		9.13	1.50	0.23	0.04		11.34	3.26	0.91	0.32
Ile	15	11	2	0	0	25	8	7	1	0
		9.83	1.91	0.35	0.07		11.34	3.78	1.22	0.55
Leu_1	14	8	3	0	0	29	12	6	0	1 (5)
		9.99	1.58	0.23	0.04		12.81	4.37	1.46	0.69
Leu_2	16	10	3	0	0	29	9	7	2	0
		10.77	1.97	0.34	0.06		12.81	4.37	1.46	0.69
Met_1^F	14	5	3	1	0	24	9	3	1	1 (4)
		9.48	1.71	0.29	0.05		11.33	3 62	1.12	0.47
Met^M	18	14	2	0	0	20	10	3	0	1 (4)
		10.64	2.51	0.56	0.15		10.99	2.90	0.73	0.23
Phe	14	10	0	0	1 (4)	24	11	3	1	1 (4)
		9.43	1.73	0.30	0.06		11.19	3.62	1.14	0.49
Ser_1	18	5	3	1	1 (4)	29	14	6	1	0
		11.48	2.35	0.46	0.10		12.96	4.37	1.44	0.67
Ser_3	17	7	3	0	1 (4)	32	13	6	1	1 (4)
		11.32	2.06	0.35	0.07		13.45	4.74	1.63	0.82
Thr	17	12	2	0	0	23	12	4	1	0
		10.06	2.13	0.43	0.10		11.16	3.46	1.04	0.41
Trp	14	6	2	0	1 (4)	23	9	2	2	1 (4)
		9.43	1.73	0.30	0.06		11.16	3.46	1.04	0.41
Tyr_1	18	8	2	2	0	23	10	3	1	1
		11.27	2.40	0.49	0.11		12.26	3.37	0.90	0.30
Tyr_2*	19	9	2	2	0	24	9	3	3	0
		11.69	2.57	0.54	0.13		12.60	3.53	0.96	0.33
Val_1	14	12	1	0	0	24	7	3	2	1 (5)
		9.43	1.73	0.30	0.06		11.19	3.62	1.14	0.49
Val_{2A}	12	12	0	0	0	26	11	5	0	1 (5)
		8.68	1.32	0.19	0.03		11.32	3.93	1.33	0.64
Val_{2B}	15	11	2	0	0	23	12	6	0	1 (5)
		9.83	1.91	0.35	0.07		11.29	3.45	1.02	0.40

* Without the modified pyrimidine ($tRNA_2^{Tyr}$).

TABLE IV

OBSERVED AND THEORETICAL DISTRIBUTION OF IDENTICAL BASE SEQUENCES FOR *E. COLI* tRNA SPECIES

For explanation of symbols see text to Table III.

tRNA	*C*					*U, ψ, T*				
	n_C	r_1	r_2	r_3	$r_{>3}$	n_U	r_1	r_2	r_3	$r_{>3}$
Ala_{1A}	23	15	1	2	0	13	7	1	1	0
		10.88	3.37	1.07	0.45		8.55	1.36	0.20	0.03
Arg_1	21	12	3	1	0	14	12	1	0	0
		11.01	3.10	0.84	0.29		9.43	1.73	0.30	0.06
Asp_1	20	9	4	1	0	17	9	4	0	0
		10.99	2.90	0.73	0.23		10.40	2.41	0.49	0.12
Gln_1	20	5	4	1	1 (4)	17	8	2	0	1 (5)
		10.78	2.94	0.76	0.25		10.24	2.34	0.51	0.13
Glu_2	25	9	1	2	2 (4)	13	6	2	1	0
		11.19	3.78	1.24	0.57		9.05	1.53	0.24	0.04
Gly_3	19	12	2	1	0	17	8	3	1	0
		10.74	2.72	0.66	0.20		10.32	2.33	0.50	0.12
His_1	17	5	3	2	0	22	10	6	0	0
		10.40	2.31	0.49	0.12		11.22	3.27	0.92	0.34
Ile	17	9	2	0	1 (4)	17	11	3	0	0
		10.40	2.31	0.49	0.12		10.40	2.31	0.49	0.12
Leu_1	22	12	1	0	1 (8)	19	9	5	0	0
		12.33	3.16	0.78	0.24		11.69	2.57	0.54	0.13
Leu_2	19	7	3	2	0	20	12	4	0	0
		11.69	2.57	0.54	0.13		11.93	2.76	0.61	0.16
Met^F_1	24	12	2	1	1 (5)	12	10	1	0	0
		11.33	3.62	1.12	0.47		8.68	1.32	0.19	0.03
Met^M	18	13	1	1	0	18	12	3	0	0
		10.64	2.51	0.56	0.15		10.64	2.51	0.56	0.15
Phe	19	6	3	1	1 (4)	16	8	4	0	0
		10.74	2.72	0.66	0.20		10.06	2.13	0.42	0.10
Ser_1	22	9	5	1	0	16	6	5	0	0
		12.23	2.95	0.68	0.19		10.82	1.96	0.33	0.06
Ser_3	26	10	4	0	2 (4)	15	13	1	0	0
		13.30	2.78	1.04	0.37		10.54	1.68	0.25	0.04
Thr	16	11	1	1	0	18	12	3	0	0
		10.06	2.13	0.43	0.10		10.55	2.53	0.58	0.16
Trp	19	7	4	0	1 (4)	17	9	4	0	0
		10.74	2.72	0.66	0.20		10.32	2.33	0.50	0.12
Tyr_1	25	9	4	1	1 (5)	16	8	4	0	0
		12.44	3.73	1.09	0.42		10.65	2.00	0.35	0.07
Tyr_2*	25	10	2	1	2 (4)	15	7	4	0	0
		12.69	3.71	1.05	0.39		10.39	1.77	0.28	0.05
Val_1	21	10	1	3	0	14	8	3	0	0
		11.01	3.10	0.84	0.29		9.43	1.73	0.30	0.06
Val_{2A}	19	13	3	0	0	17	9	4	0	0
		10.83	2.40	0.65	0.19		10.40	2.31	0.49	0.12
Val_{2B}	16	10	3	0	0	20	6	7	0	0
		10.13	2.11	0.42	0.09		10.99	2.90	0.73	0.23

* Without the modified pyrimidine ($tRNA_2^{Tyr}$).

TABLE V

DISTRIBUTION OF PURINE BASES IN THE ANTICODON ARM OF *E. COLI* tRNA SPECIES

The upper lines give the observed distribution of purine bases in the anticodon arm (positions 33–48 according to Fig. 1). The lower lines indicate the calculated or theoretical distribution of purine bases assuming a random base distribution in the whole molecule of tRNA (ref. 1).

tRNA	*Anticodon*		*Total number or residues*		
	A	*G*	*A*	*G, I*	*n (−CCA)*
Ala_{1A}	2	5	10	27	72
	2.39	6.22			
Arg_1	3	5	14	24	73
	3.26	5.59			
Asp_1	2	5	10	27	74
	2.30	6.20			
$Gln_{1,2}$	2	5	14	20	72
	3.30	4.72			
Glu_2	1	5	13	22	73
	3.03	5.12			
Gly_3	3	5	12	25	73
	3.07	5.67			
His_1	3	5	13	22	74
	2.99	5.05			
Ile	4	5	15	25	74
	3.45	5.74			
Leu_1	3	5	14	29	84
	2.83	5.87			
Leu_2	3	6	16	29	84
	3.36	5.87			
Met_1^F	4	4	14	25	75
	3.17	5.67			
Met^M	6	2	18	20	72
	4.25	4.72			
Phe	5	5	14	24	72
	3.30	5.67			
Ser_1	3	5	18	29	85
	3.60	5.80			
Ser_3	3	5	17	32	90
	3.21	6.04			
Thr	3	6	16	23	73
	3.73	5.36			
Trp	4	4	14	23	73
	3.26	5.36			
Tyr_1	4	4	18	23	82
	3.73	4.77			
Tyr_2	5	4	19	24	84
	3.85	4.86			
Val_1	4	4	14	24	73
	3.26	5.59			
Val_{2A}	3	5	12	26	74
	2.76	5.97			
Val_{2B}	3	5	15	23	74
	3.45	5.28			

Tables III and IV present the distribution of identical base sequences observed (upper line) and calculated (lower line) without considering the common 3′-terminal trinucleotide. The hypothesis of a random distribution of bases is acceptable (χ^2 test) for the great majority of *E. coli* tRNAs except for $tRNA_F^{Met}$ which has an excess of G and C compared to A and U. There is a random sequence distribution of identical bases, except for $tRNA_1^{Ser}$, characterized by a significant cluster of A, and for $tRNA_1^{Gln}$ with regard to C. Table V notes the distribution of purine bases in the anticodon arm. Contrary to some yeast tRNAs[1], *E. coli* tRNAs do not differ significantly in purine distribution in this region compared with their average distribution in the whole molecule.

Counter-current distribution

Fig. 3 shows the absorbance profile of *E. coli* tRNA species fractionated by CCD, over 160 transfers at 15°, in the solvent system PMB (18.6% 2-butoxy ethanol,

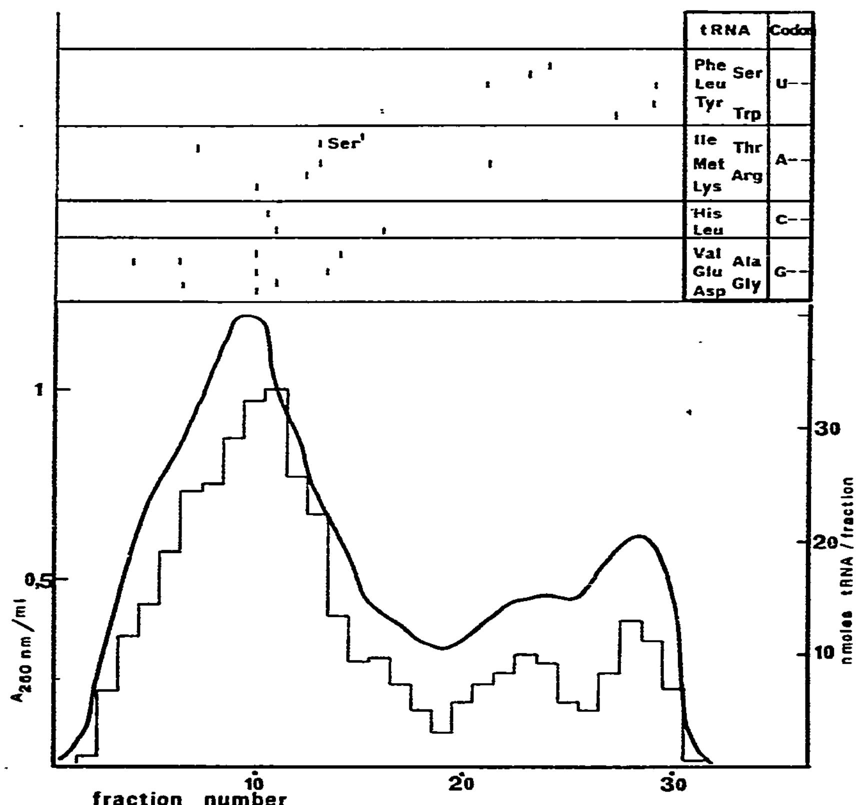
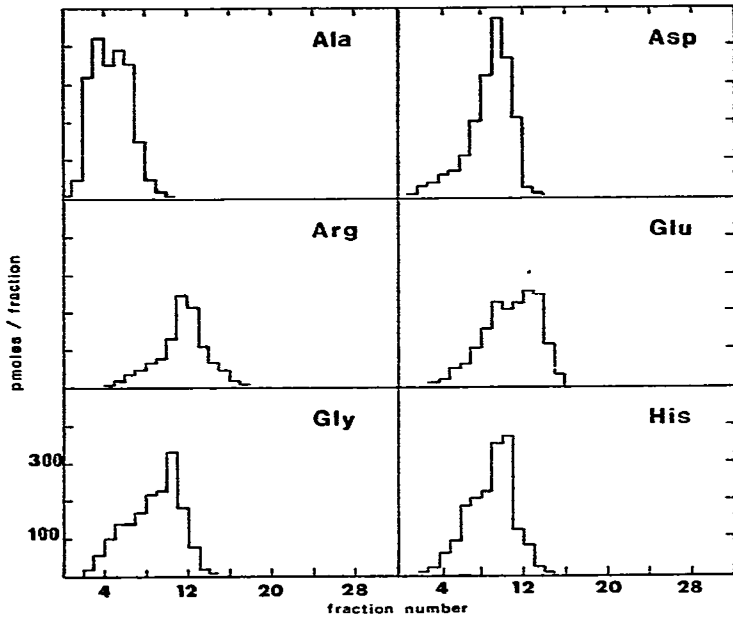


Fig. 3. CCD of *E. coli* tRNA. 600 $A_{260\ nm}$ units of *E. coli* B tRNA are distributed over 160 transfers at 15° in the salt–solvent system PMB (1.50 potassium phosphate buffer pH 7.0, 2-methoxy ethanol and 2-butoxy ethanol) with 18.6% 2-butoxy ethanol and 0.8 m*M* $MgCl_2$. —, Absorbance of tRNA at 260 nm in the upper phase; —, distribution curve of 16 individual tRNA species titrated by acylation in nmoles per fraction (one fraction is composed of the tRNA content of five distribution elements); ı, indication of the position of the maximum concentration of the isoacceptor species arranged according to their corresponding codons[50].



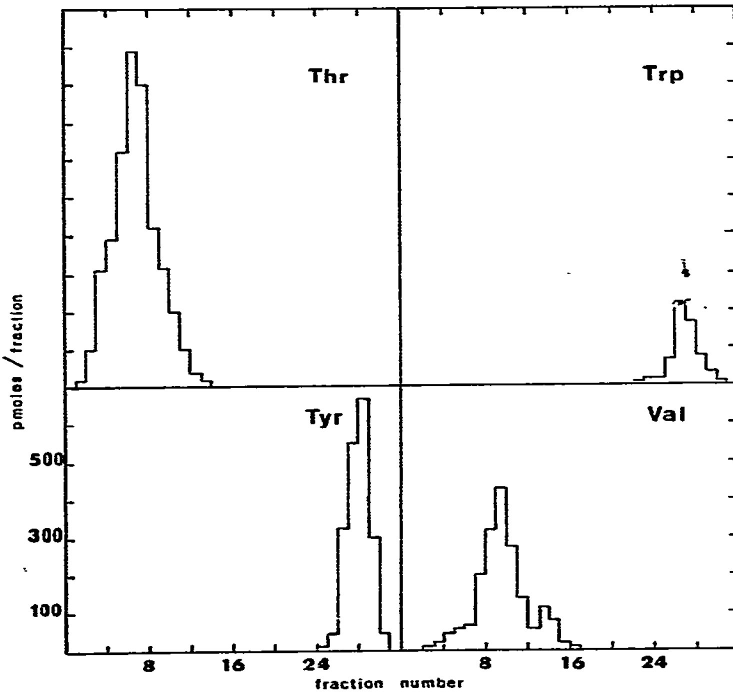


Fig. 4.

(Continued on p. 102)

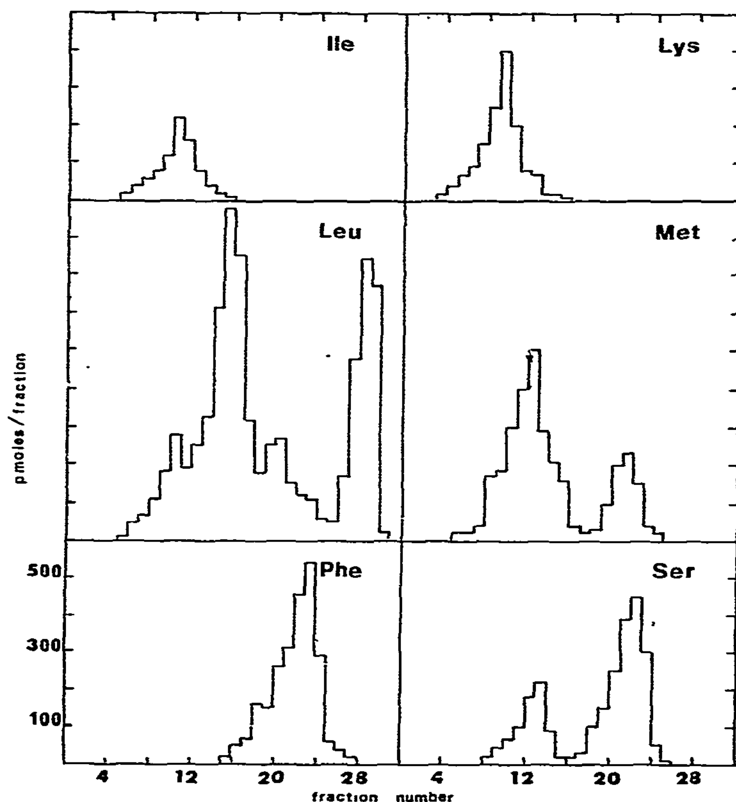


Fig. 4. Analysis of *E. coli* tRNA species fractionated by CCD. Conditions of the CCD in the PMB solvent system are indicated on Fig. 3. After 160 transfers, the contents of five distribution tubes are pooled, 5 ml of 2-methoxy ethanol is added; the solution is equilibrated in the cold and the lower phase discarded whereas the organic top phase is extracted with diethylether, dialyzed and lyophilized. tRNA from each of the 32 fractions was dissolved in 2.0 ml of 5 m*M* sodium acetate buffer (pH 4.7), 1 m*M* $MgCl_2$ and acylated according to Chavancy *et al.*[11] with homologous enzymes. The order of mobility of isoaccepting species is given according to the nature of the first base of the codon, indicated in decreasing order of polarity: A, U, G and C according to Wehrli and Staehelin[50].

0.8 m*M* $MgCl_2$). The mobility order of individual tRNA species is indicated on the diagram of Fig. 4. It is analogous to the elution order found by Wehrli and Staehelin[50], who did partition chromatography with the PEB solvent system described by Muench and Berg[51]: 1.25 *M* potassium phosphate buffer (pH 6.88), 2-ethoxy ethanol and 2-butoxy ethanol containing 1% of triethylamine. Figs. 3 and 5 suggest a direct relationship between the polarity of tRNA and its coding properties. Fig. 5 shows the mobility of *E. coli* tRNA fractionated by CCD over 970 transfers in the PFI solvent system[52] as used by Goldstein *et al.*[53] (1.7 *M* sodium phosphate buffer (pH 6.0), formamide and isopropanol). Formamide plays an analogous role to that of 2-methoxy ethanol in the PMB system; its addition increases the partition coefficient of nucleic acid. Isopropanol, less polar, has a similar effect to that of 2-butoxy ethanol, which decreases partition coefficient[12,54]. Table VI summarizes partition coefficients values available and based on CCDs in the solvent systems PMB and PFI.

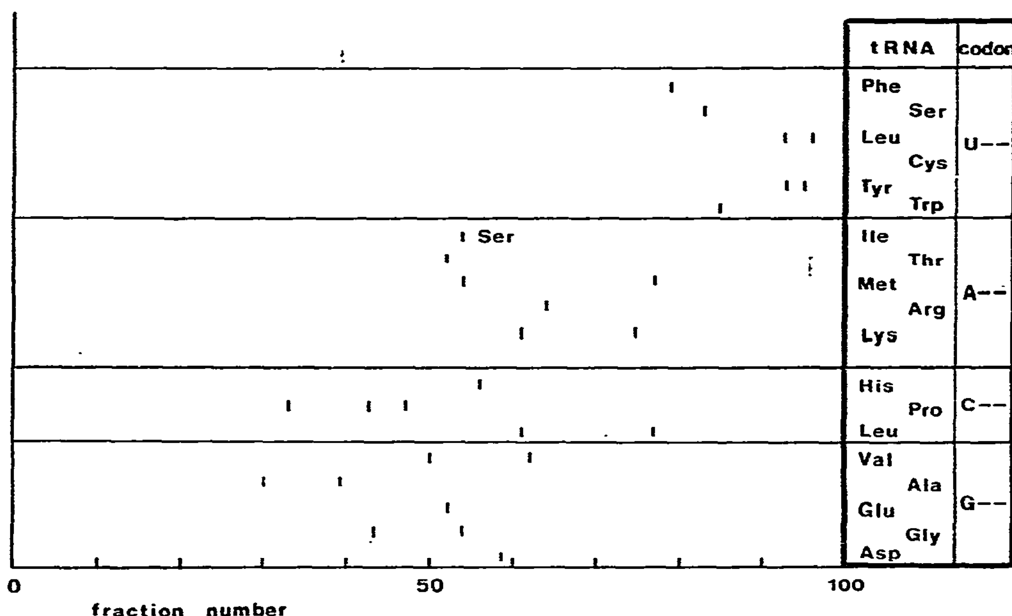


Fig. 5. Order of mobility of *E. coli* tRNA species fractionated by CCD over 970 transfers in the salt–solvent system PFI (phosphate buffer, formamide and isopropanol) according to Goldstein *et al.*[53].

Mobility law of E. coli tRNA species

As we have shown for oligoribonucleotides[2], yeast tRNAs[1] and ribosomal RNA fragments[3], the partition coefficient K increases with an enrichment in adenosine residues expressed as A/(A + G) or A/R and also with an increase in the ratio of purine/pyrimidine nucleotides, R/Y. For a salt–solvent system S studied at temperature T, one may write the following relationship:

$$\log K_{(S,T)} = c\, \mathrm{A}/(\mathrm{A} + \mathrm{G}) \cdot \mathrm{R}/\mathrm{Y} + d$$

where c and d are constants dependent on the solvent system used and the characteristics of the nucleic compound (anticodon loop polarity, helix content, chain length and conformation). By neglecting the presence of inosine, the mobility of which is close to that of guanosine, and by considering the pyrimidine nucleotides as a whole (see Table VII for cytidine and uridine values of K), this relationship is reduced to

$$\log K_{(S,T)} = c\, \mathrm{A}/\mathrm{Y} + d\,.$$

This formula is only valid for a similar total number of nucleotides.

The semi-logarithmic plot of the partition coefficients of *E. coli* tRNAs as a function of A/Y nucleotide composition (Fig. 6 for the PMB system and Fig. 7 for the PFI system) shows a linear relationship for tRNAs according to the polarity of their anticodon loops. The position of each point appears to be the result of two structural factors: A/Y composition for tRNA species having closely similar anti-

TABLE VI

PARTITION COEFFICIENTS OF *E. COLI* tRNA SPECIES FRACTIONATED BY COUNTER-CURRENT DISTRIBUTION IN SALT–SOLVENT SYSTEMS

Partition coefficients have been calculated using the formula $K = r/n - r$ where $r \leqslant t$ (r = element of the distribution apparatus corresponding to the maximum concentration of an isoacceptor species, n = total of transfers for a distributor with t elements or tubes).

tRNA	*PFI (ref. 52)*	*PFI (ref. 59)*	*PMB (ref. 54)*	*Suggested fit with a tRNA of known sequence*
Alanine	0.39		0.13	
	0.58		0.22	Ala_{1A}
Arginine	1.50		0.69	$Arg_{1,2}$
Aspartate	0.50			
	1.22		0.46	Asp_1
Glutamate			0.49	Glu_1
	0.96		0.73	Glu_2
Glycine	0.72		0.25	Gly_2
	1.00		0.52	$Gly_{1,3}$
Histidine	1.13		0.50	His_1
Isoleucine		0.47		
		0.79		
		1.00	0.88	Ile_1
Leucine	1.32		0.52	
	2.58	1.35	1.00	Leu_1
			1.91	Leu_2
	9.0	9.0	7.1	
Lysine	1.32			
	2.31		0.46	Lys
Methionine	0.96		0.68	Met^F
	2.58		1.90	Met^M
Phenylalanine	2.70		3.00	Phe
Proline	0.45			
	0.66			
	0.79			
Serine	1.04	0.61	0.74	Ser_3
	3.54	1.44	2.60	Ser_1
Threonine	0.92		0.31	Thr
Tryptophane	4.00		5.4	Trp
Tyrosine	6.7			Tyr_1
	7.9		9.7	Tyr_2
Valine	0.89		0.46	Val_{2A}
	1.38		0.76	Val_1

codon loop polarity (neighbouring d values) and, for a nearly identical overall composition, variable polarity of the anticodon loop (d variable over an interval Δd).

Group 1 (hydrophilic anticodon loop) comprises $tRNA^{Ile}$, $tRNA^{Lys}$, $tRNA^{Met}_{M}$, $tRNA^{Ser}_{3}$ containing in the 3′-position of the anticodon a polar N-(purin-6-yl carbamoyl)-threonine riboside (t^6A), $tRNA^{Thr}$ having a methylated t^6A or mt^6A, $tRNA^{Ala}_{1A}$, $tRNA^{Ser}_{1}$ and $tRNA^{Val}_{1}$ carrying a 5-oxyacetic uridine acid in the wobble position[55]. In a semi-logarithmic diagram, correlation lines can be drawn as shown in Figs. 6 and 7. The partition coefficient value for $tRNA^{Thr}$ has not been taken into account. It should be noted that because of the presence at the 3′-end of the anticodon of the

TABLE VII

PARTITION COEFFICIENTS OF SOME MODIFIED NUCLEOSIDES

Determinations are done at 20° in the PMB solvent system with 20% 2-butoxy ethanol[60].

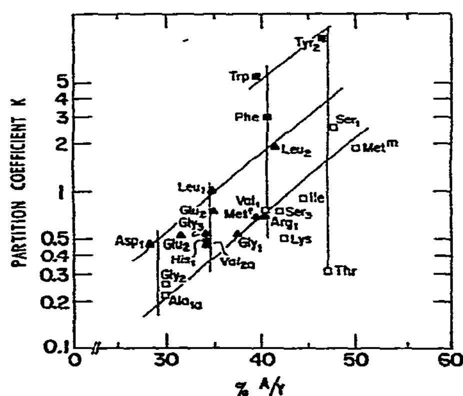
Nucleoside	*K*	*log K*
N^6-Dimethyl adenosine	13.8*	1.14*
Adenosine	3.75	0.57
N^1-Methyl adenosine	0.70	−0.15
N^2-Dimethyl guanosine	11.5	1.06
1-Methyl guanosine	1.75	0.24
Guanosine	1.40	0.15
Inosine	1.10	0.04
N^7-Methyl guanosine	0.37	−0.43
Thymidine	1.90	0.28
Uridine	1.15	0.06
5-Methyl cytidine	1.08	0.03
Cytidine	1.04	0.02
Pseudouridine	0.66	−0.19
N^3-Methyl cytidine	0.30	−0.52

* Estimated according to the log *K* of corresponding modified base and the negative contribution of ribose residue ($\Delta F_{rib} = -0.09$).

lipophilic nucleoside ms^2i^6A, $tRNA_1^{Ser}$ might be expected to be located in the group 3 with apolar anticodon loops, but the strong polarity of the nucleoside oac^5U prevails over the lipophilic effect of the modified adenosine.

Group 2 (neutral anticodon loop) contains a 2-methyl adenosine in the 3′-position of the anticodon except a 1-methyl guanosine for $tRNA_{1,2}^{Leu}$. It should be noted that $tRNA_{1,3}^{Gly}$ and $tRNA_{2A}^{Val}$ do not have a modified nucleoside in that loop.

Group 3 (lipophilic anticodon loop) consists of $tRNA^{Phe}$, $tRNA^{Trp}$ and $tRNA^{Tyr}$,



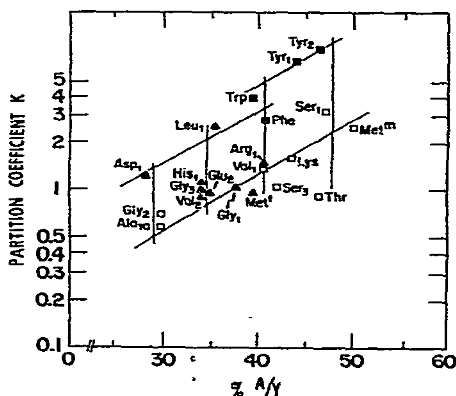


Fig. 6. Semi-logarithmic plot indicating the change of partition coefficients as a function of the total nucleotide composition of *E. coli* tRNA species distributed by CCD in the salt–solvent system PMB (see Fig. 3 and Tables I and VI). □, tRNA group with polar anticodon loop; ▲, tRNA group with neutral anticodon loop; ■, tRNA group with lipophilic anticodon loop.

Fig. 7. Semi-logarithmic plot giving the change of the partition coefficients as a function of the total nucleotide composition of *E. coli* tRNA species distributed by CCD in the salt–solvent system PFI according to Goldstein *et al.*[53] (see Fig. 5 and Tables I and VI). For legend see Fig. 6.

and is characterized by a 2-methylthio N^6-isopentenyl adenosine at 3′-end of the anticodon.

The strong effect exerted by the modified nucleosides of the exposed anticodon loop on the mobility of tRNA macromolecules can be shown by considering the polarity order of modified purine and pyrimidine nucleosides. From data given in Table VII and from the chromatographic mobilities of modified adenosines in the partition solvent system used by Rogg *et al.*[56], we suggest the following decreasing order of polarity: t^6A, mt^6A, m^7G, m^1A, I, G, m^1I, m^1G, m^2G, A, m^2A, m^6A, m_2^2G, m_2^6A, i^6A, ms^2i^6A. The mobility order of m^1I and m^2G with respect to m^1G is uncertain. Most of the modified purine nucleosides are located in the wobble position and from the 3′-end to the anticodon (positions 40 and 43 of the generalized cloverleaf structure of Fig. 2). For modified pyrimidine nucleosides mainly located in positions 38 and 40, we propose the order: oac^5U, m^3C, ψ, C, m^5C, U, ac^4C, s^4U, mam^5s^2U, Cm, Um. The contribution of a positive charge carried by the methyl group in the nucleosides m^1A, m^3C and m^7G can be quantitated: $\log K = -0.70 \pm 0.02$ for purine and -0.54 for cytidine nucleoside in the PMB solvent system. For extreme polarities of purine nucleosides K values could change by a factor 30. Our series includes some modified residues (m^1A, m^3C, m_2^2G) present only in eukaryotic tRNA species.

For similar overall composition of tRNAs expressed as A/Y ratio, the increasing order of K values can be correlated with the variable polarity of the anticodon loop expressed as $\Delta d = \log K_1 - \log K_2$ ($K_1 > K_2$). The three *E. coli* tRNA species (Ala_{1A}, Gly_2 and Asp_1) with the lowest A/Y ratio ($29.4 \pm 0.8\%$) have anticodon loops with different polarities: a polar oac^5U nucleoside for $tRNA_{1A}^{Ala}$ and an unknown modified uridine for $tRNA_2^{Glu}$ in contrast to the non-polar loop of $tRNA_1^{Asp}$ containing a modified guanosine, the Q nucleoside having a 7-deazaguanosine nucleus with a cyclopentenediol side chain (Kasai *et al.*[57]), and a m^2A residue ($\Delta d = 0.32$ in both solvent systems).

For tRNA species having the composition $A/Y = 34.5 \pm 0.4\%$ (Val_{2A}, Gly_3, His_1, Glu_2 and Leu_1) one observes an analogous difference in partition coefficients ($\Delta d = 0.34$ in PMB and 0.45 in PFI systems).

The remarkable set of eight tRNA species with a close overall composition ($A/Y = 40.7 \pm 1.2\%$) is arranged in order of increasing mobility: $tRNA^{Lys}$, $tRNA_F^{Met}$, $tRNA_1^{Arg}$, $tRNA_3^{Ser}$, $tRNA_1^{Val}$, $tRNA_2^{Leu}$, $tRNA^{Phe}$ and $tRNA^{Trp}$ ($\Delta d = 0.90$ in PMB and 0.62 in PFI systems). Their mobility differences depend upon the differences in lipophilicity of their anticodon loops: anticodon loops with polar residues such as oac^5U in $tRNA_1^{Val}$ or t^6A in $tRNA^{Lys}$ and $tRNA_3^{Ser}$ and the lipophilic residue ms^2i^6A found in $tRNA^{Phe}$ and $tRNA^{Trp}$. The presence of an apolar 2′-O-methyl cytidine (Cm) in the anticodon loop of $tRNA^{Trp}$, in contrast to the relatively polar pseudouridine (ψ in position 38) in $tRNA^{Phe}$, might play a role in the higher mobility of $tRNA^{Trp}$. In addition, we may consider that $tRNA_2^{Leu}$ is 15% longer than $tRNA_1^{Arg}$ with comparable anticodon polarity. According to Brönsted relationship (Brönsted[58]), one may expect that an increase in molecular weight of a macromolecule induces an increase in the partition coefficient.

The mobility differences are much greater for the last five tRNA species: $A/Y = 47.1 \pm 2.9\%$ for $tRNA^{Thr}$, $tRNA^{Ile}$, $tRNA_M^{Met}$, $tRNA_1^{Ser}$ and $tRNA^{Tyr}$ ($\Delta d = 1.51$ in PMB and 0.94 in PFI systems). The lower K value of $tRNA_1^{Ser}$ results from the antagonistic effects of polar oac^5U and lipophilic ms^2i^6A. In the same way

we can explain the higher mobility of $tRNA^{Met}_{M}$ relative to $tRNA^{Ile}$ by substitution in the wobble position of a non-polar ac^4C with a guanosine. The peculiar mobility of $tRNA^{Thr}$ in both salt–solvent systems remains much more difficult to interpret.

DISCUSSION

In a given salt–solvent system S at the temperature T, the partition coefficient K is a function of five structural parameters: the nucleotide composition, the sequence, the molecular weight or the chain length, the helix content and the conformation.

Nucleotide composition. The nucleotide composition indicated by the ratio A/Y for nucleic acids of similar chain length. Our general formula does not take into account the particular contribution of modified nucleosides or their location in a region available to the solvent. As we have shown, the presence or absence of lipophilic or hydrophilic residues in the anticodon loop greatly changes the partition coefficient of tRNA species having close overall compositions. On the other hand, tRNAs with different compositions can have similar chromatographic mobilities if their anticodon loop polarities compensate the effect of the whole composition on the K value. An increasing lipophilicity of tRNA (increasing A/Y ratio) corresponds to an increasing polarity of the anticodon loop for the following couples: $tRNA^{Glu}_{2}$–$tRNA^{Ser}_{3}$, $tRNA^{Gly}_{3}$–$tRNA^{Lys}$, $tRNA^{Leu}_{1}$–$tRNA^{Ile}$ in the PMB system and $tRNA^{Met}_{F}$–$tRNA^{Thr}$ in the PFI system for intermediate K values; $tRNA^{Phe}$–$tRNA^{Ser}_{1}$ and $tRNA^{Leu}_{3}$–$tRNA^{Met}_{M}$ for high K values.

Sequence. Garel *et al.*[2] have shown that different sequences have no measurable effect on K, except when there is a non-random distribution of bases and clustering of purines or pyrimidines in an accessible region of the macromolecule (Garel and Mandel[1]). Tables III–V show that bases in *E. coli* tRNA species so far studied, including the bases in the anticodon arm, are randomly distributed.

Molecular weight or the chain length. According to the Brönsted relationship (Brönsted[58]), an increase in molecular weight brings about an increase in the partition coefficient for a similar nucleotide composition. tRNA species with higher chain length ($tRNA^{Leu}$, $tRNA^{Ser}$ and $tRNA^{Tyr}$) but with differing anticodon loop polarities are found among the most lipophilic.

Helix content. Double strandedness strongly increases the partition coefficient, as has been shown by Albertsson[61] with native and denatured DNA and various polynucleotides. Since the helix content of *E. coli* tRNA species varies only within very narrow limits ($54 \pm 3\%$), we may consider its contribution to tRNA mobility nearly identical for all species.

Conformation. It is known that in high ionic strength, the tertiary structure of tRNA is more compact (Fresco *et al.*[62]). Melting temperature or Tm values of various fractions, determined in presence of both high ionic strength (potassium phosphate buffer 2.6 M for the lower phase, 0.21 M for the upper phase in the PMB solvent system with 18.6% 2-butoxy ethanol at 15° according to Garel *et al.*[60]) and 2-methoxy ethanol, are much higher than in low ionic strength medium[63]. The presence of hydrophilic organic solvents and their interactions with nucleotides located at the surface of a tRNA macromolecule do not produce noticeable denaturation. Hanlon and Major[64] observed that the polyadenylic acid solubilized in slightly acidic polyethylene glycol maintains its secondary structure. Our results show that the main factor affecting

the chromatographic mobility of tRNA species is the polar or non-polar character of the anticodon loop. Our analysis indicates that this region remains accessible to the solvent in all tRNA species. This conformation in salt–solvent systems is consistent with that determined by X-ray crystallographic data[9,10].

When both the partition coefficient *K* value and overall composition A/Y ratio are determined for a tRNA of unknown sequence, its position on our semi-logarithmic diagram allows predictions for modified nucleosides in the anticodon loop, as suggested for $tRNA^{Ala}$ and $tRNA^{Tyr}$ species from silk gland of *Bombyx mori* L.[12].

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