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Small Angle X-Ray Study of Alanine Transfer Ribonucleic Acid and Bulk Yeast Transfer Ribonucleic Acid

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ABSTRACT: Small-angle X-ray diffraction measurements were performed upon alanine transfer ribonucleic acid (t-RNA) and bulk yeast transfer t-RNA in 0.1 *M* tris(hydroxymethyl)aminomethane hydrocholoride at pH 7.0. The radius of gyration, axial ratio, volume, and surface per unit volume were evaluated. The "cloverleaf" model of Holley does not allow an axial ratio as large as that observed, 4.0:1. The "hairpin" model of Brown and Zubay provides the best agreement with these data. The dimensions of alanine specific and bulk yeast t-RNA are very similar, which strongly implies that all of the yeast t-RNA's have approximately the same molecular conformation in solution. Addition of 0.1 *M* $MgCl_2$ to the buffer resulted in aggregation of both alanine and bulk yeast t-RNA.

The biological synthesis of proteins is remarkable for its specificity. The recognition role is performed by amino acid transfer ribonucleic acids (t-RNA's), each of which carries a particular amino acid to template RNA in the ribosomes.[2a] The discovery of transfer RNA by Hoagland, Zamecnik, and Stephenson[2b] was followed by a period of mounting interest in the elucidation of the molecular structures of the amino acid specific t-RNA's. Holley, *et al.*,[3] were the first to determine the primary structure (nucleotide sequence) of a specific t-RNA, that for alanine. Subsequently the complete sequences have been reported for two serine t-RNA's,[4] one specific for tyrosine,[5] and one for phenylalanine.[6] A given amino acid may have more than one t-RNA, so the number of possible t-RNA's exceeds 20. Nevertheless, it appears likely that the complete sequences of many of these will be determined by chemical means within the next few years.

The ability to assume a specific tertiary structure appears to be a major factor in the biological activity of the t-RNA's. It might be hoped that application of the criterion of maximal base pairing to a known nucleotide sequence would lead directly to a single tertiary structure of greatest stability. However, the longest sequence of complementary bases in alanine t-RNA contains only five base pairs, so the helical regions are either short or imperfect due to looping out of the chains. This circumstance has led to the postulation of a number of possible tertiary structures having approximately the same helical content, but differing widely in molecular conformation. Among these we cite only two containing a single helical region, the "hairpin" model of Brown and Zubay[7] and the triple helix of Guschlbauer,[8] and the "cloverleaf" model of Holley, *et al.*,[3] consisting of four helical regions flexibly connected about a common center. Further progress was possible as more sequences became known, since these were found to have certain features in common. Each of the known sequences could be arranged in some variant of the "cloverleaf" pattern, thereby providing circumstantial evidence favoring this model. A more direct assessment of the merits of these proposed structures is possible through study of the molecular conformation in solution by low angle X-ray diffraction.

Materials and Methods

Dr. R. W. Holley kindly provided samples of bulk yeast t-RNA, and of alanine t-RNA isolated therefrom by countercurrent distribution.[3] Both were studied in 0.1 *M* tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) at pH 7.0. The solutions were stored under refrigeration, and prior to use they were centrifuged at 12,000 rpm for 20 min using a Spinco Model L preparative centrifuge. Concentrations were determined by ultraviolet absorption.

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(1) National Aeronautics and Space Administration Research Fellow, 1963–1966.

(2) (a) A. S. Spirin in "Progress in Nucleic Acid Research," Vol. 1, J. N. Davidson and W. E. Cohn, Ed., Academic Press, New York, N. Y., 1963, pp 301–345; (b) M. B. Hoagland, P. C. Zamecnik, and M. L. Stephenson, *Biochim. Biophys. Acta*, **24**, 215 (1957).

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(7) G. L. Brown and G. Zubay, *J. Mol. Biol.* **2**, 287 (1960).

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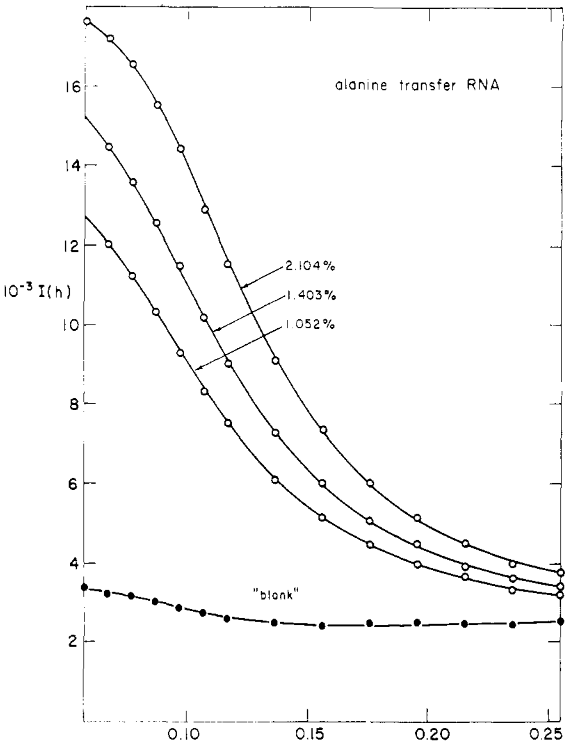

Figure 1. Intensity *vs.* $h = (4\pi/\lambda) \sin \theta$ for three concentrations of alanine t-RNA. The lowest curve is the "blank" scattering.

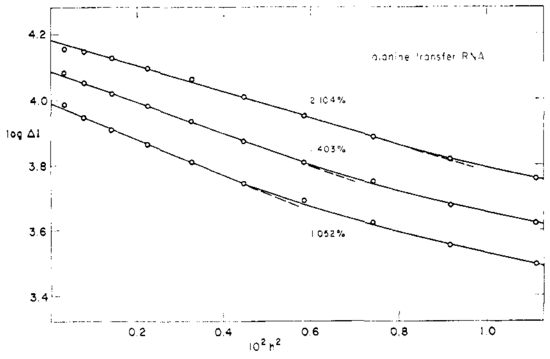

Figure 2. Guinier plot for three concentrations of alanine t-RNA in 0.1 *M* Tris-HCl buffer at pH 7.0.

Diffraction measurements were performed using a Kratky camera and Cu Kα radiation effected by the Ross balanced filter technique.[9] A special stainless steel sample cell having 0.15-mil mylar windows was constructed so that 10 mg of t-RNA sufficed for the study of two solvent systems, tris buffer and buffer plus magnesium ion. Intensities were measured relative to a Lupolene standard of known scattering power,[10] and 10^4 counts were accumulated at each angle. The scattering curves were desmeared using a Fortran program of Brumberger and Kent.[11]

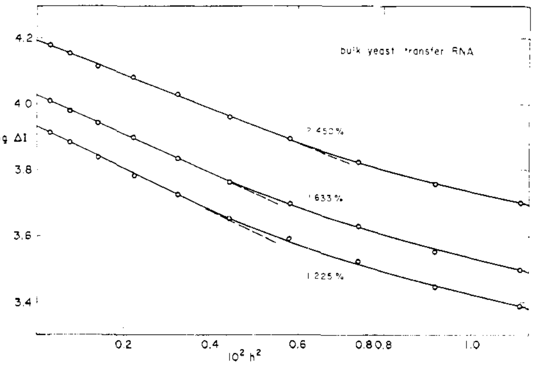

Figure 3. Guinier plot for three concentrations of bulk yeast t-RNA in 0.1 *M* Tris-HCl buffer at pH 7.0.

Results

Specific Extinction Coefficients. Values obtained for bulk yeast t-RNA using a 1-cm path quartz cell were 190 at 256 mμ and 226 at 206 mμ. The former may be compared with two values reported for 260 mμ, 219 by Prown and Zubay,[7] and 204 Tissières.[12] The specific extinction coefficient of alanine t-RNA was assumed to be the same as that for bulk yeast t-RNA.

Small Angle X-Ray Measurements. The scattering angle 2θ is related to the vertical displacement *m* (centimeters) of the camera according to eq 1. For all

$$m/a = \tan 2\theta \cong 2\theta \qquad (1)$$

measurements the sample to detector distance, *a*, was 21.0 cm, and the entrance and detector slit widths were 80 and 380 μ, respectively. These parameters were selected to optimize the experimental arrangement for a resolution of 700 Å. Figure 1 shows the scattering curves for three concentrations of alanine t-RNA and, for comparison, the "blank" scattering due to solvent, cell windows, etc., which must be subtracted from the total scattered intensity of the solutions to obtain the excess intensity, ΔI. Here the angular variable is $h = (4\pi/\lambda) \sin \theta$, were λ is the wavelength of the radiation. Guinier plots are illustrated for alanine t-RNA and bulk yeast s-RNA in Figures 2 and 3, respectively. We note that these plots are linear over a moderate range of scattering angles, and that the linearity extends to the smallest angles investigated. Vainshtein and Feigin[13] and Dembo, Sosfenov, and Feigin[14] observed scattering curves for s-RNA which decreased at the lowest angles, indicating interparticle interference. Since our data do not exhibit this behavior, we may apply the theory of independent particle scattering due to Guinier[15] to relate the initial slope of each curve to the radius of gyration, *R*, according to eq 2. A tilde (~) is used to designate slit

$$\ln \tilde{I}(h) = \ln \tilde{I}(0) - h^2\tilde{R}^2/3 \qquad (2)$$

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(9) P. A. Ross, *Phys. Rev.*, **28**, 425 (1926).

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TABLE I

ALANINE TRANSFER RNA (0.1 *M* TRIS-HCl, pH 7.0)

c, g/100 cc	$10^{-4}I(0)$	$\tilde{Q}$	Lim $h^3\Delta\tilde{I}(h)$	$\tilde{R}$, Å	R, Å	10^3V, Å^3	S/V, Å^{-1}
2.104	1.58	28.2	2.10	16.6	17.4	22.1	0.293
1.403	1.42	21.3	1.58	18.2	19.2	26.3	0.295
1.052	1.17	16.6	1.22	19.2	20.5	28.0	0.292
0.0	...	...	...	21.7 + 9.4^a	23.3 ± 0.4^a	34.3 ± 0.5^a	0.293 ± 0.002^a
0.956 (0.1 *M* MgCl$_2$)	1.15	14.1	0.99	...	...	32.2	0.281

^{a} Extrapolated values.

TABLE II

BULK YEAST TRANSFER RNA (0.1 *M* TRIS-HCl, pH 7.0)

c, g/100 cc	$10^{-4}I(0)$	$\tilde{Q}$	Lim $h^3\Delta\tilde{I}(h)$	$\tilde{R}$, Å	R, Å	10^3V, Å^3	S/V, Å^{-1}
2.450	1.90	24.1	1.72	19.0	21.0	31.2	0.282
1.633	1.39	16.8	1.26	20.4	21.9	32.6	0.299
1.225	1.13	13.3	1.02	21.0	22.4	33.4	0.303
0	...	...	...	23.0 ± 0.4^a	23.9 ± 0.4^a	35.6 ± 0.5^a	0.300 ± 0.003^a
2.227 (0.1 *M* MgCl$_2$)	...	24.0	1.58	...	...	...	0.263
1.114 (0.1 *M* MgCl$_2$)	...	11.2	0.67	...	...	...	0.240

^{a} Extrapolated values.

smeared parameters. Slit smeared and desmeared values, $\tilde{R}$ and R, are given in Table I for alanine t-RNA, while those for bulk yeast s-RNA appear in Table II.

It is well known that certain divalent ions, particularly Mg^{2+}, are essential for the biological activity of the t-RNA's. Therefore several concentrations of alanine t-RNA and bulk yeast s-RNA were examined in solutions which were 0.1 *M* in Tris-HCl and 0.1 *M* in MgCl$_2$. In all cases the Guinier plots showed upward curvature in the innermost portion, indicating aggregation. The curvature was not as pronounced in the case of alanine t-RNA, but even here a reliable value for the radius of gyration could not be obtained. Millar and Steiner[16] have recently reported a more thorough investigation of aggregation of *Escherichia coli* s-RNA in the presence of magnesium ion.

According to the treatment of Porod,[17] if the assumption of a uniform electron density within the scattering particle is valid the outermost portion of the scattering curve should obey a $1/h^3$ law (or $1/h^4$ for desmeared intensities). Therefore, a plot of $h^3\Delta\tilde{I}(h)$ *vs.* h^3 should appear as a horizontal line. However, Luzzati, Witz, and Nicolaieff[18] have suggested that there may be a contribution to the intensity in the outer portion of the scattering curve due to internal structure within the scattering particle, in which case the expression for the scattering intensity at large values of h becomes eq 3.

$$\Delta\tilde{I}(h) = K_1/h^3 + K_2 \qquad (3)$$

Hence, a plot of $h^3\Delta\tilde{I}(h)$ *vs.* h^3 may give rise to an ascending straight line. The slope of the line yields K_2, which must be subtracted from the scattering curve before continuing with the analysis. Figure 4 shows this plot for alanine t-RNA. The horizontal portion extending over a considerable range of scattering angles indicated that the assumption of uniform electron density within the molecule is valid for the range of scattering angles investigated.

Other parameters derivable from the X-ray scattering depend upon calculation of the invariant, $\tilde{Q}$, defined by Porod[17] as

$$\tilde{Q} = \int_0^\infty h\Delta\tilde{I}(h)\,\mathrm{d}h \qquad (4)$$

This parameter may be used to evaluate the volume, V, of the scattering molecule and the surface per unit volume, S/V (eq 5 and 6). Here $I(0)$ is desmeared

$$V = 4\pi^2 I(0)/\tilde{Q} \qquad (5)$$

$$S/V = 2\pi v_1 \frac{\lim\,[h^3\Delta\tilde{I}(h)]}{\tilde{Q}} \qquad (6)$$

intensity extrapolated to zero scattering angle, λ is the wavelength of the radiation, 1.54 Å, and v_1 is the volume fraction of the solvent. The limit appearing in eq 6

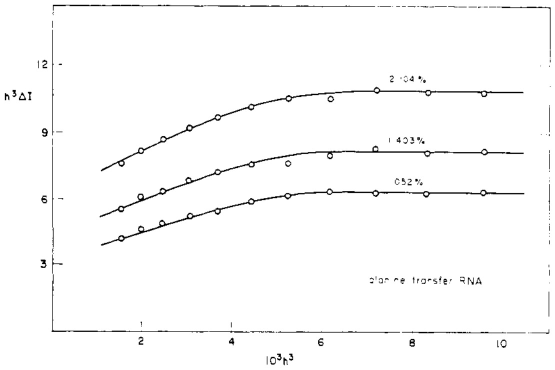

Figure 4. Data for alanine t-RNA in 0.1 *M* Tris-HCl buffer plotted according to eq 3 to check for internal structure contributions.

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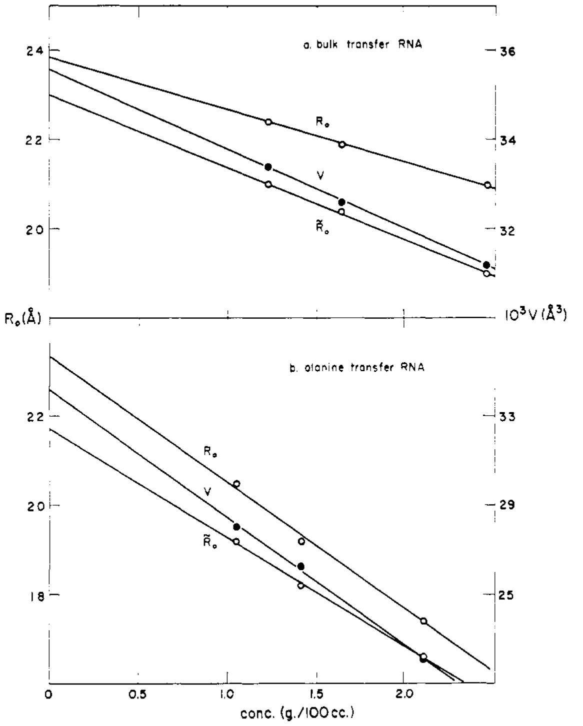

Figure 5. Extrapolations of V, $\tilde{R}$, and R to infinite dilution to eliminate interparticle interference for (a) bulk yeast t-RNA and (b) alanine t-RNA.

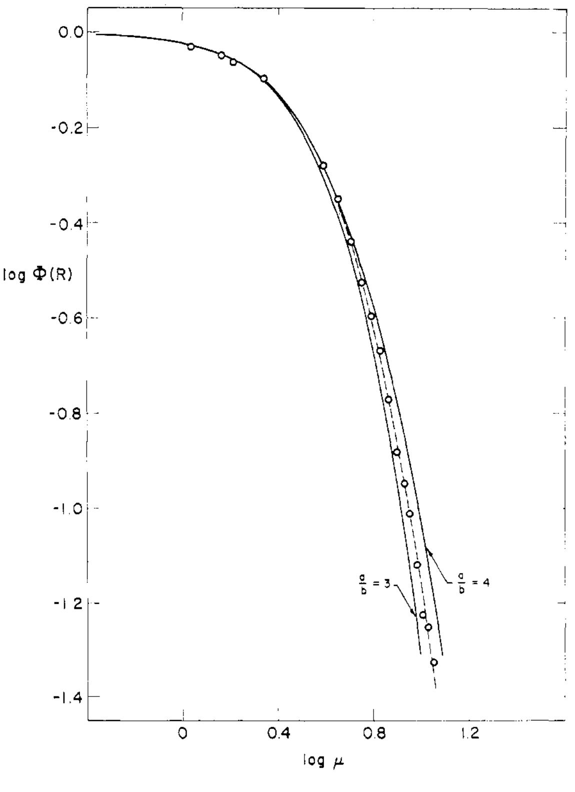

Figure 6. Comparison of the observed scattering curve for alanine t-RNA (dashed) with the theoretical scattering curves for ellipsoids having axial ratios 3 and 4.

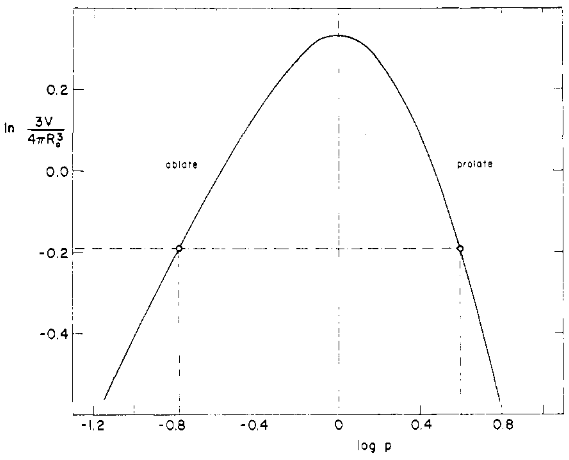

Figure 7. Particle shape analysis curve for ellipsoids using the method of Ritland, Kaesberg, and Beeman.

is with respect to increasing h. Values deduced for V and S/V are listed in Tables I and II. In order to eliminate interparticle interference effects completely, the experimental values of $\tilde{R}$, R, and V measured at different concentrations were extrapolated to infinite dilution (Figure 5) to obtain the corresponding entries in Tables I and II. For both alanine t-RNA and bulk yeast s-RNA the S/V ratio was independent of concentration, in agreement with theoretical prediction.[17]

The molar volume of unhydrated bulk yeast s-RNA can be calculated from the molecular weight, 26,600, and the partial specific volume, 0.531 cc/g.[19] If this is subtracted from the expermental volume, 2.14×10^4 cc/mol, one finds that there is 0.3 g of H_2O/g of s-RNA. This may be compared with the value 0.4 g of H_2O/g of s-RNA deduced by Luborsky and Cantoni[20] from purely hydrodynamic considerations.

The intermediate portion of the scattering curve (beginning beyond the Guinier region) contains information concerning the particle shape. In Figure 6 the desmeared scattering curve for alanine t-RNA (1.403%) is compared with the theoretical scattering curves for ellipsoids of revolution having semiaxis ratio 3 and 4. From this comparison the semiaxis ratio is found to be approximately 3.5. The corresponding curve (not shown) for the 1.052% alanine t-RNA yielded essentially the same result. Another procedure for shape analysis suggested by Ritland, Kaesberg, and Beeman[21] and Luzzati, Witz, and Nicolaieff[22] is based upon ratios of the experimental parameters which are independent of the dimensions of the particular molecule under investigation. For example, the relation given in eq 7 may be derived for rotational ellipsoids, where p is the ratio of the two semiaxes.

$$\frac{3V}{4\pi R^3} = \frac{p}{[(p^2 + 2)/5]^{3/2}} \qquad (7)$$

From the plot of log $(3V/4\pi R^3)$ *vs.* log p shown in Figure 7 one obtains two values of p, corresponding to

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TABLE III
PARTICLE SHAPE PARAMETERS

Sample	p	a, Å	b, Å	V, Å
Alanine t-RNA	3.98 (prolate)	49.0	12.3	31,100
	$(6.02)^{-1}$ oblate	50.5[a]	8.4	90,000
Bulk yeast s-RNA	4.04	50.3	12.5	33,000
	$(6.30)^{-1}$	50.6[a]	8.1	85,900

[a] a is always defined as the major semiaxis.

an oblate or a prolate ellipsoid. Values of p, the semiaxis lengths a and b, and V obtained in this manner for alanine t-RNA and bulk yeast s-RNA are listed in Table III. Comparison with the experimental volumes given in Tables I and II clearly eliminates the oblate ellipsoid, and thus this procedure leads to a prolate ellipsoid of revolution having axial ratio 4.0. We believe this value is more reliable, since the former procedure depends heavily on the outer portion of the scattering curve where the diffracted intensity is low. Thus we conclude that the equivalent particle shape best representing alanine t-RNA is a prolate ellipsoid having semiaxes 49.0 and 12.3 Å, while for bulk yeast s-RNA the semiaxes are 50.3 and 12.5 Å. These smooth ellipsoids would have surface-to-volume ratios of 0.20 Å^{-1}, while the experimental values were 0.29 and 0.30 Å^{-1} for alanine t-RNA and bulk yeast s-RNA, respectively. The S/V ratio is sensitive to surface irregularities,[18] and the observed difference is just what would be anticipated for an imperfect helical structure containing portions of the chains looped out of the helix.

Discussion

Of the models discussed at the outset, the "hairpin" structure proposed by Brown and Zubay is in best agreement with our observations. This would have over-all dimensions approximately 100 × 27 Å, in reasonable agreement with those of our equivalent scattering particle, 100 × 25 Å. The experimental radius of gyration and axial ratio are both too large to fit the model of Guschlbauer. The "cloverleaf" model could assume a variety of shapes, since the four helical regions are flexibly connected, but even the most extended of these would not have an axial ratio in excess of 2.5, so our data are not compatible with this model.

Most of the available experimental evidence is in agreement with our conclusion that the t-RNA molecules are rather elongated. Tumanyan, Esipova, and Kiselev[23] deduced an axial ratio for bulk yeast s-RNA between 3.8 and 4.0 from a photographic low angle study. In this analysis they appear not to have used their uncertain value for the radius of gyration, R = 32 ± 5 Å, which is in poor agreement with our result. Kiselev and Kiselev[24] had earlier reported an electron microscope study of rat liver s-RNA and yeast s-RNA which indicated the molecules to be 80–100 Å long and 20–25 Å wide. Furthermore, the axial ratios deduced indirectly from hydrodynamic studies have consistently been rather large. For example, an axial ratio of 7 was reported for yeast s-RNA,[25] 6 for rabbit liver s-RNA,[20] and 5 for wheat germ s-RNA.[26]

A significantly different result has recently appeared in a preliminary report of a small angle X-ray study of bulk yeast s-RNA by Lake and Beeman.[27] Their radius of gyration, R = 23.5 ± 0.25 Å, is in excellent agreement with our value, 23.9 ± 0.4 Å. However, they state that their measured scattering intensities in the shape region could not be represented by theoretical ellipsoid or cylinder scattering curves, and they favor two possible variants of the "cloverleaf" model having an axial ratio in the range 2.0–2.5. We can offer no explanation for this disagreement beyond the suggestion that s-RNA may have two possible conformations of approximately the same free energy.

Our low-angle results carry the further implication that all of the yeast t-RNA's have approximately the same molecular conformation in solution. The slight difference found for the dimensions of alanine t-RNA and bulk yeast s-RNA may be explained by the fact that alanine t-RNA has a somewhat shorter chain length (78 nucleotides) than average. This result would also be anticipated from gel chromatographic studies[28, 29] of s-RNA, which show that alanine t-RNA is among the last to be eluted from the column. Further confirmatory evidence is provided by Tumanyan, Esipova, and Kiselev,[23] who observed similarly shaped scattering curves for bulk yeast s-RNA and for samples which had been enriched in valine or alanine.

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(24) N. A. Kiseley and L. L. Kiselev, *ibid.*, **141,** 900 (1961).

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