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Structure of the Ribotrinucleoside Diphosphate Codon UpUpC Bound to $tRNA^{Phe}$ from Yeast

A Time-dependent Transferred Nuclear Overhauser Enhancement Study

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The structure of the ribotrinucleoside diphosphate UpUpC, the codon for phenylalanine, bound to yeast $tRNA^{Phe}$ in solution is elucidated using time-dependent proton–proton transferred nuclear Overhauser enhancement measurements to determine distances between bound ligand protons. The glycosidic bond and ribose conformations are low anti and 3′-*endo*, respectively, typical of an *A*-RNA type structure. The main chain torsion angles are all within the range of those expected for *A*-RNA but small differences from those in conventional *A*-RNA 11 result in a special structure with a larger rotation per residue (40 to 45° compared to 32·7° in *R*-RNA 11) and almost perfect stacking of the bases. These two structural features, which are similar to those found in the anticodon triplet of the monoclinic crystal form of $tRNA^{Phe}$, can account for the known greater stability of the codon–anticodon complex relative to an equivalent double helical RNA trimer with a conventional *A*-RNA structure.

1. Introduction

In recent years a considerable amount of structural data has been accumulated on a number of isolated tRNA species both in the crystal state from high-resolution X-ray diffraction studies (for reviews, see Kim, 1981; Wright, 1982) and in solution from ^1H n.m.r.† studies, principally involving the use of the nuclear Overhauser effect to demonstrate the proximity of imino protons in space

† Abbreviations used: n.m.r., nuclear magnetic resonance; NOE, nuclear Overhauser effect; TRNOE, transferred nuclear Overhauser effect; D, deuterium; p.p.m., parts per million.

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(Johnston & Redfield, 1978; Roy & Redfield, 1983; Hare & Reid, 1982; Heerschap *et al.*, 1982,1983*a*,*b*). However, there are no direct structural data available as yet on codon–tRNA complexes. Previous work in this area has concentrated mainly on exploring the thermodynamics (Uhlenbeck *et al.*, 1970; Eisenger *et al.*, 1971; Pongs *et al.*, 1973; Eisenger & Gross, 1975; Grosjean *et al.*, 1976; Pörschke & Labuda, 1982; Labuda & Pörschke, 1983) and kinetics (Yoon *et al.*, 1975; Labuda & Pörschke, 1980) of codon–anticodon interactions, and on observing conformational changes induced by codon binding, by means of fluorescence probes (Schwarz *et al.*, 1976; Robertson *et al.*, 1977), chemical modification (Wagner & Garrett, 1979) and shifts in the proton magnetic resonances (Geerdes *et al.*, 1980*a*,*b*). Given that the codon–anticodon complex plays a central role in the translation of the genetic message during protein synthesis, it is clear that a complete understanding of this process will require a knowledge of the structure of the codon–anticodon complex. As a first step in this direction, we have made use of time-dependent proton–proton transferred nuclear Overhauser enhancement measurements (Clore & Groneneborn, 1982,1983*a*) to determine intra- and internucleotide interproton distances between protons of the ribotrinucleoside diphosphate UpUpC, the codon for the amino acid phenylalanine, in its complex with yeast $tRNA^{Phe}$ in solution. From these distances, the structure of UpUpC bound to $tRNA^{Phe}$ is solved by model building.

2. Experimental

UpUpC was synthesized from the suitably protected nucleosides according to the 1-hydroxybenztriazole phosphotriester approach (Van der Marel *et al.*, 1981), deblocked in a series of 3 steps and purified by anion-exchange chromatography on Sephadex A-25. The purity of the isolated oligoribonucleotide was controlled by high pressure liquid chromatography on an anion-exchange column. The purified product could be digested completely by ribonuclease T_2 to give uridine 3′-monophosphate and cytidine in a ratio of 2 : 1.

Yeast $tRNA^{Phe}$ was purified to homogeneity by chromatography initially on benzoylated DEAE-cellulose followed by elution from a 21 mm × 250 mm column of ODS-Hypersil containing dynamically bound trioctylmethylammonium chloride (Bischoff *et al.*, 1983). No attempt was made to remove tightly bound magnesium ions from the purified $tRNA^{Phe}$.

The samples for ^1H n.m.r. were freeze-dried extensively from 99·6% D_2O and finally dissolved in 99·96% D_2O buffer containing 10 mM-phosphate, 0·02 mM-EDTA, 500 mM-KCl, pH* 6·6 (meter reading uncorrected for the isotope effect on the glass electrode). All glassware was heated to 200°C for 4 h before use to inactivate possible traces of ribonuclease.

^1H n.m.r. spectra were recorded at 500 MHz on a Bruker AM 500 spectrometer. Spectra were recorded with a 90° observation pulse, an acquisition time of 0·5 s (spectral width 8·2 kHz, 8 K data points) and a relaxation delay of 1 s. The time-dependent TRNOEs were observed by directly collecting the difference free induction decay by interleaving 16 transients after saturation for a set time of a given resonance, with 16 transients of off-resonance irradiation (applied for the same length of time), negating the memory between 16 transient cycles. Before Fourier transformation the free induction decay values were multiplied by an exponential equivalent to a line broadening of 1 Hz. Chemical shifts are expressed relative to 4,4-dimethylsilapentane-1-sulphonate.

Model building was carried out manually using Nicholson skeletal models at a scale of 1 Å to 1 cm.

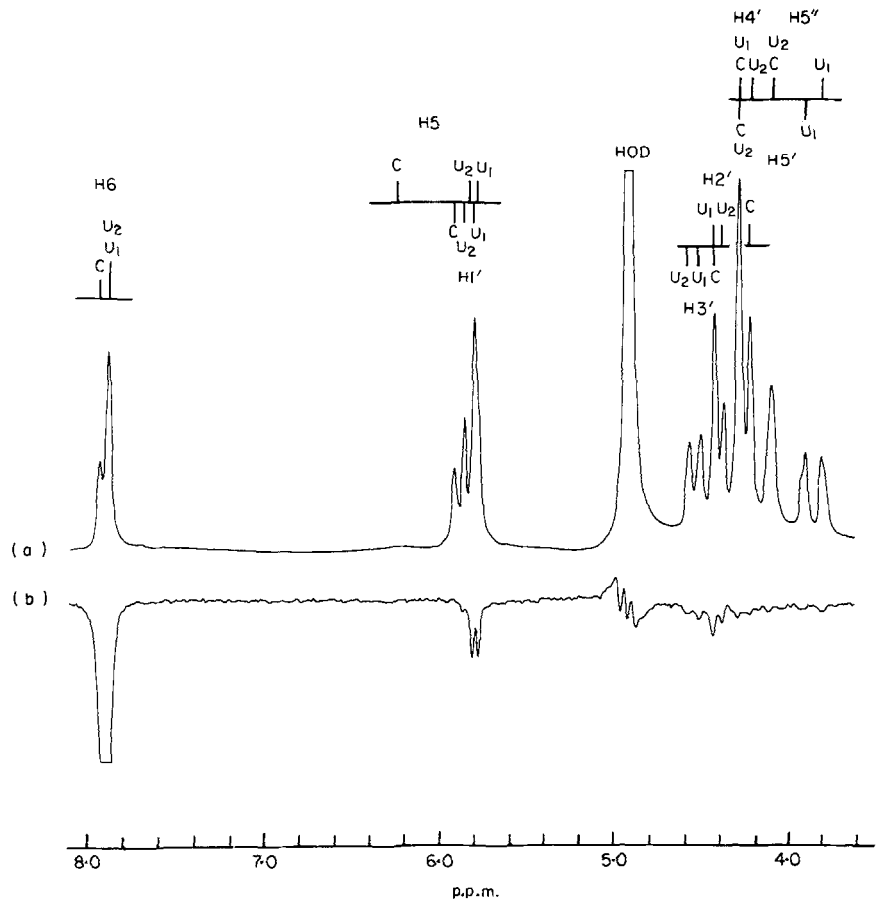

FIG. 1. The 500 MHz ^{1}H n.m.r. spectrum of 28 mM-UpUpC in the presence of 0·5 mM-tRNA^{Phe} corresponding to a ratio of free to bound UpUpC of 55. (a) Reference spectrum (160 transients) together with the assignments of the proton resonances (Gronenborn *et al.*, 1983); (b) TRNOE difference spectrum (160 transients on-resonance minus 160 transients off-resonance pre-irradiation) following pre-saturation for 0·4 s of the averaged U_1(H6)/U_2(H6) resonance at 7·89 p.p.m. (the gain in (b) is 4 times that in (a)).

The experimental conditions are: 28 mM-UpUpC, 0·5 mM-tRNA^{Phe}, 500 mM-KCl, 0·02 mM-EDTA, 10 mM-potassium phosphate (pH* 6·6).

3. Results and Discussion

Figure 1(a) shows the 500 MHz ^{1}H n.m.r. spectrum of 28 mM-UpUpC in the presence of 0·5 mM-tRNA^{Phe} corresponding to a ratio of free to bound UpUpC of 55, together with the assignments of the UpUpC proton resonances. The binding of UpUpC to tRNA^{Phe} is weak ($K_a \lesssim 2 \times 10^3$ M^{-1} and $k_{off} \gtrsim 2000$ s^{-1}; Labuda & Pörschke, 1980), so that chemical exchange between free and bound UpUpC is fast on both the chemical shift scale and the cross-relaxation rate scale. Consequently, only a single set of average exchange broadened ligand resonances is seen. The broadening of the UpUpC resonances at this large ratio of free to

bound ligand is much more extensive than one would expect for a $tRNA^{Phe}$–UpUpC complex of $M_r \sim 24{,}000$ and indeed, one resonance, the H5 resonance of C_3, is broadened almost beyond detection. Such extensive broadening is indicative of significant aggregation of $tRNA^{Phe}$–UpUpC complexes and is discussed in more detail below†.

The TRNOE involves the extension of NOE measurements to exchanging systems, making use of chemical exchange between the free and bound states of the ligand to transfer magnetic information concerning cross-relaxation between bound ligand protons from the bound state to the free state (Clore & Gronenborn, 1982,1983*a*,*b*). Thus, in this case the TRNOE experiment involves irradiating each average ligand resonance in turn and observing the effect on the intensities of the other average ligand resonances. In the case of free UpUpC (28 mM), no NOE could be observed between any pair of proton resonances at irradiation times of less than one second. (This is not surprising, as one would expect $\omega\tau_c$, where ω is the Larmor frequency and τ_c the correlation time, to be close to 1 for a molecule of the size of UpUpC, such that the cross-relaxation rates, and hence the steady state NOEs, have values very close to zero.) Consequently, the initial slope of the time development of the TRNOE, $N_i(j)$, observed on the averaged ligand resonance of proton i following irradiation of the averaged ligand resonance of proton j, is simply given by:

$$\left.\frac{dN_i(j)}{dt}\right|_{t=0} = -(1-a)\sigma_{i_B j_B}, \tag{1}$$

where a is the mole fraction of free ligand and $\sigma_{i_B j_B}$ is the cross-relaxation rate between the bound ligand protons i_B and j_B (Clore & Gronenborn, 1983*a*). In this manner, cross-relaxation rates between pairs of bound ligand protons can be determined, thus enabling distance ratios between any two such pairs of protons to be calculated from the equation:

$$r_{i_B j_B}/r_{k_B l_B} = (\sigma_{k_B l_B}/\sigma_{i_B j_B})^{1/6}, \tag{2}$$

assuming the same correlation time for the two interproton distance vectors.

A TRNOE difference spectrum obtained on irradiating the U_1(H6)/U_2(H6) average ligand resonance at 7·89 p.p.m. for 0·4 second is illustrated in Figure 1(b) clearly showing large negative TRNOEs ($\sim -16\%$) on the U_2(H5) and U_1(H5) resonances and smaller negative TRNOEs ($\lesssim -5\%$) on the U_2(H3′), U_1(H3′), U_1(H2′), U_2(H2′), U_2(H5′), U_2(H5″), U_1(H5′) and U_1(H5″) resonances. The time development of these TRNOEs is shown in Figure 2 and the absence of lag phase in all cases is indicative of direct (first order) TRNOEs. The cross-relaxation rates between pairs of bound UpUpC protons determined from the observed initial slopes of the time-dependent TRNOEs using equation (1) are summarized in Table 1, together with the interproton distances calculated from them using equation (2) and the distance (2·46 Å) and cross-relaxation rate between the H5

† Note that free UpUpC (i.e. in the absence of $tRNA^{Phe}$) does not appear to aggregate at the high concentration (28 mM) employed in these experiments, as evidenced by the observation that the linewidths of a 0·5 mM and 28 mM solution of UpUpC are identical and equally narrow.

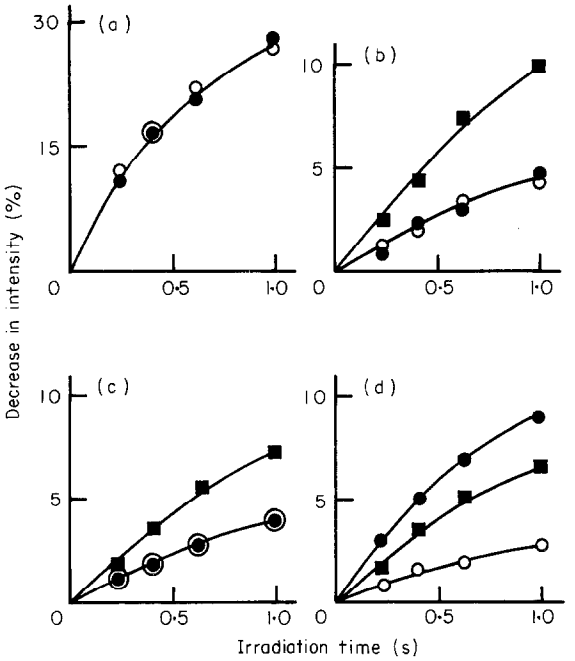

FIG. 2. Time dependence of the TRNOEs observed following irradiation of the averaged $U_1(H6)/U_2(H6)$ resonance of UpUpC in the presence of tRNA^{Phe} with a ratio of free to bound ligand of 55. The experimental conditions are as in the legend to Fig. 1. (a) ●, $U_2(H5)$; ○, $U_1(H5)$. (b) ■, $U_1(H2')$; ○, $U_2(H1')$; ●, $U_2(H2')$. (c) ■, $U_1(H3')$; ●, $U_1(H5')$; ○, $U_1(H5'')$. (d) ●, $U_2(H5')$; ■, $U_2(H3')$; ○, $U_2(H5'')$.

and H6 protons of the U base as an internal reference. It should be noted that the cross-relaxation rates have been calculated on the assumption that the ratio of free to bound ligand was 55; that is to say, on the assumption that each anticodon has a molecule of UpUpC bound to it. If for some reason, however unlikely, saturation of the anticodon by UpUpC is incomplete, the calculated cross-relaxation rates would be smaller than the real ones by a factor $1/x$, where x is the extent of saturation. This, however, would have no effect on the calculation of interproton distance ratios and distances, as these are calculated from ratios of cross-relaxation rates.

A further consideration to be borne in mind is the effect and extent of any possible non-specific binding. Non-specific binding of UpUpC to tRNA^{Phe} has not been detected in previous studies with ratios of UpUpC to tRNA^{Phe} up to 160 and concentrations of UpUpC up to 2·5 mM (Labuda & Pörschke, 1980,1983; Pörschke & Labuda, 1982). Moreover, there is only one partially complementary sequence in tRNA^{Phe}, namely $G_{20}A_{21}$ in the D loop, to which UpUpC could potentially bind, in addition to binding to the anticodon triplet $G_{m34}A_{35}A_{36}$. It seems highly probable that the binding of UpUpC to the $G_{20}A_{21}$ sequence is very weak ($K \lesssim 2\ M^{-1}$) on three counts: (1) the equilibrium association constant ($\sim 1{\cdot}5 \times 10^3\ M^{-1}$) for the binding of UpUpC to the anticodon triplet in intact

TABLE 1

Cross-relaxation rates for UpUpC bound to tRNA^{Phe} determined from time-dependent TRNOE measurements on a solution of 28 mM-UpUpC in the presence of 0·5 mM-tRNA^{Phe}, together with the interproton distances calculated from them

A. *Intranucleotide*

	U_1		U_2		C_3	
	$\sigma_{i_B j_B}(s^{-1})$	$r_{i_B j_B}(Å)$	$\sigma_{i_B j_B}(s^{-1})$	$r_{i_B j_B}(Å)$	$\sigma_{i_B j_B}(s^{-1})$	$r_{i_B j_B}(Å)$
Intrasugar						
H1′–H2′	11·2	2·90	11·2	2·90	9·5	2·98
H1′–H4′	5·0	3·32	3·4	3·55	5·6	3·26
H2′–H3′	15·1	2·76	11·8	2·88	10·1	2·95
H3′–H4′	[a]		1·7	3·97	[a]	
H3′–H5′	3·9	[b]	[a]		[a]	
H3′–H5″	3·9	[b]	7·3	3·12	14·6	2·78
H4′–H5′	9·5	[b]	[a]		[a]	
H4′–H5″	9·0	[b]	17·4	2·70	[a]	
H5′–H5″	60·5	[b]	[a]		[a]	
Sugar–base						
H1′–H6	[a]		2·8	3·66	2·24	3·80
H2′–H6	2·6 [c]	~ 3·7 [c]	2·8	3·66	5·6	3·26
H3′–H6	7·3	3·12	5·6	3·26	5·6	3·26
H5′–H6	2·2	[b]	6·7	3·16	9·0	3·01
H5″–H6	2·2	[b]	2·2	3·80	2·2	3·80
Base–base						
H5–H6	30·2	2·46	30·2	2·46	[d]	

B. *Internucleotide*

		U_1pU_2		U_2pC_3	
5′ nucleotide	3′ nucleotide	$\sigma_{i_B j_B}(s^{-1})$	$r_{i_B j_B}(Å)$	$\sigma_{i_B j_B}(s^{-1})$	$r_{i_B j_B}(Å)$
H1′	H6	[a]		Lag	
H2′	H6	15·1 [c]	2·8 [c]	8·9	3·01
H3′	H6	[a]		2·8	3·66
H2′	H1′	3·9	3·46	1·1	4·26
H2′	H5′	[a]		8·4	3·05
H3′	H5′	[a]		9·5	2·98

The experimental conditions are as in the legend to Fig. 1. The relative errors in the values of the cross-relaxation rates are $\lesssim \pm 15\%$. Interproton distances are calculated from eqn (2) using the distance (2·46 Å) and cross-relaxation rate between the H5 and H6 protons of the U bases as an internal reference. Assuming an error of $\pm 0{\cdot}05$ Å in the value of the reference distance (calculated on the basis of standard bond lengths and angles), the error in the values of the calculated distances is $\lesssim \pm 0{\cdot}15$ Å.

[a] Individual TRNOEs between these protons cannot be extracted due to signal overlap resulting in the superposition of TRNOEs arising from several pairs of protons.

[b] The distance between the H5′ and H5″ protons calculated on the basis of standard bond lengths and angles is 1·78 Å. The correlation time calculated for the H5′–H5″ distance vector of U_1 from eqn (3) using the standard distance and measured cross-relaxation rate is $3{\cdot}4 \times 10^{-8}$ s compared to a value of $\sim 1{\cdot}2 \times 10^{-7}$ s calculated from the distance (2·46 Å) and cross-relaxation rate between the H5 and H6 protons of the U bases. Moreover, the cross-relaxation rates between the H3′ and H5′, H4′ and H5′ and H6 and H5′ protons are approximately equal to the corresponding cross-relaxation involving the H5″ proton. These observations are best explained by free rotation about the C4′–C5′ bond of U_1.

[c] Because the U_1(H6) and U_2(H6) resonances are superimposed, the intranucleotide TRNOE

$tRNA^{Phe}$ is at least three orders of magnitude larger than that expected for the binding of the two equivalent complementary ribotrinucleoside diphosphates, which is too weak to measure (Jaskunas *et al.*, 1968; Pongs *et al.*, 1971; Borer *et al.*, 1974; Freier & Tinoco, 1975; Labuda & Pörschke, 1980; D. Pörschke, personal communication); (2) the stronger binding of UpUpC to the anticodon can be attributed to structural features specific to the anticodon loop such as the 3′-stacked conformation, the U-turn hydrogen bond and the ability of the anticodon triplet to stack almost perfectly (Yoon *et al.*, 1975; Jack *et al.*, 1976); and (3) hydrogen bonding of UpUpC to the $G_{20}A_{21}$ sequence would be expected to be particularly unfavourable. The latter arises from two factors: (1) steric hindrance preventing access of UpUpC due to the fact that the G_{20} and A_{21} residues are located at the junction of the D loop and stem and are buried within the tertiary structure of $tRNA^{Phe}$; and (2) some of the atoms of G_{20} and A_{21} that could potentially hydrogen bond to the UpC segment of UpUpC are already involved in hydrogen-bonding interactions with other residues of $tRNA^{Phe}$, namely through the G_{20}(N2H)—G_{22}(O2′), A_{21}(N6H)—C_{48}(O1′) and the A_{21}(N1)—U_8(O2′H) hydrogen bonds (Jack *et al.*, 1976). Thus, if we assume that the equilibrium constant for the binding of UpUpC to the $G_{20}A_{21}$ sequence of the D arm is $\lesssim 2\ M^{-1}$, then the occupancy of the $G_{20}A_{21}$ site would be less than 5% at the concentration of UpUpC (28 mM) employed. Even an occupancy of 20%, corresponding to $\sim 15\%$ of the total bound UpUpC, would have no effect on the structural conclusions, as the initial slopes can be measured only within a relative error of $\lesssim \pm 15\%$.

(a) *Association of $tRNA^{Phe}$–UpUpC complexes*

The cross-relaxation rate $\sigma_{i_B j_B}$ between two bound ligand protons i_B and j_B is given by:

$$\sigma_{i_B j_B} = \frac{1}{10} \frac{\gamma^4 \hbar^2}{r_{i_B j_B}^6} \left(\tau_c - \frac{6\tau_c}{1+4\omega^2\tau_c 2} \right), \tag{3}$$

where γ and $\hbar$ have their usual meanings (Solomon, 1955; Kalk & Berendsen, 1976). Using the distance (2·46 Å) and measured cross-relaxation rate between the H5 and H6 protons of the U bases of bound UpUpC, the value for the correlation time τ_c of bound UpUpC is $\sim 1{\cdot}2 \times 10^{-7}$ second. On the basis of the Stokes–Einstein equation, this value corresponds to a complex of $M_r \sim 220{,}000 \pm 50{,}000$.

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between the U_1(H2′) and U_1(H6) protons cannot be separated *a priori* from the internucleotide TRNOE between the U_1(H2′) and U_2(H6) protons, and only a single cross-relaxation rate of $17{\cdot}7\ s^{-1}$ can be measured. However, on the basis of the other intranucleotide interprotons distances determined for U_1, the distance $r_{H2'\,H6}$ for U_1 is estimated as $\sim 3{\cdot}7$ Å from model building. This distance corresponds to a cross-relaxation rate of $2{\cdot}6\ s^{-1}$. Hence, the cross-relaxation rate between the U_1(H2′) and U_2(H6) protons can be estimated to be $15{\cdot}1\ s^{-1}$, which yields a distance of $\sim 2{\cdot}8$ Å.

[d] TRNOEs involving the H5 proton of C_3 cannot be measured as the C_3(H5) proton resonance is broadened almost beyond detectability.

That is to say, to a multi-complex system consisting of 9 ± 2 $tRNA^{Phe}$–UpUpC complexes. (This calculation assumes complete saturation of the anticodon by UpUpC; as the correlation time τ_c for the interproton vector $i-j$ is directly proportional to the cross-relaxation rate σ_{ij} under conditions where $\omega\tau_c \gg 1$ (as in the present case), the effect of incomplete saturation of the anticodon by UpUpC on the calculated value of τ_c would be the same as that on σ_{ij}: namely, τ_c, and hence the estimated size of the aggregate, would be underestimated by a factor $1/x$, where x is the extent of anticodon saturation.)

Equilibrium sedimentation studies have demonstrated that $tRNA^{Phe}$–UpUpC complexes undergo self-association (Pörschke & Labuda, 1982; Labuda & Pörschke, 1983); these data are consistent with dimer formation, although higher-order aggregates cannot be excluded and, indeed, would be difficult to observe given the value of the apparent dimerization constant (6×10^4 M^{-1}) and the concentrations of $tRNA^{Phe}$ employed ($\lesssim 50\ \mu M$). Moreover, from the UpUpC concentration dependence of self-association, it was shown that association occurs only between $tRNA^{Phe}$ molecules to which UpUpC is bound and not between a $tRNA^{Phe}$–UpUpC complex and a $tRNA^{Phe}$ molecule without UpUpC bound (Pörschke & Labuda, 1982). Thus, association through hydrogen bonding between the anticodon $G_{m34}A_{35}A_{36}$ of one $tRNA^{Phe}$ molecule and the complementary $T_{54}\Psi_{55}C_{56}$ sequence of the TΨC loop of another $tRNA^{Phe}$ molecule can be excluded. Indeed, such a possible mechanism would be highly unlikely, given that triplet codon-induced tRNA association has also been observed for $tRNA^{Lys}$ and $tRNA^{Met}$, where the conserved TΨC sequence is not complementary to the anticodon (Labuda & Pörschke, 1983). The present finding of very large $tRNA^{Phe}$–UpUpC aggregates at high concentrations of $tRNA^{Phe}$ (0·5 mM) confirms and extends the observations by equilibrium sedimentation studies and lends further support to the possible functional significance of such association in terms of the stabilization of the translation complex with multiple tRNA molecules after the recognition of contiguous codons (Labuda & Pörschke, 1983). It should be noted that self-association of $tRNA^{Phe}$–UpUpC complexes will be critically dependent on the $tRNA^{Phe}$–UpUpC concentration, so that the large multi-complex aggregate found here is easily accounted for by the high $tRNA^{Phe}$ concentration used.

It should be noted that a correlation time of $3{\cdot}4 \times 10^{-8}$ second is calculated from the distance (1·78 Å) and cross-relaxation rate between the H5′ and H5″ protons of U_1. The reason for this shorter correlation time is simply due to free rotation about the C4′–C5′ bond of U_1 in bound UpUpC, as evidenced by the observation that the cross-relaxation rate between the U_1(H5′) and U_1(H6) protons is the same as that between the U_1(H5″) and U_1(H6) protons, and similarly that the cross-relaxation rate between the U_1(H5′) and U_1(H3′) protons is equal to that between the U_1(H5″) and U_1(H3′) protons (see Table 1).

Finally, it should be stressed that the distance ratios and distances extracted from the TRNOE data, and hence the structure deduced for UpUpC bound to the anticodon of $tRNA^{Phe}$, is independent of both the extent and mechanism of self-association of $tRNA^{Phe}$–UpUpC complexes for the reasons discussed in the previous section: namely, that the interproton distance ratios and distances are calculated from ratios of cross-relaxation rates.

(b) *Structure of UpUpC bound to tRNA^{Phe}*

The structure of UpUpC bound to tRNA^{Phe} was determined by model building on the basis of the intra- and internucleotide interproton distances given in Table 1 using the principles described previously (Clore & Gronenborn, 1983*b*). These distances are consistent with a unique conformation described by the structural parameters given in Table 2. It can be seen that the conformations of the glycosyl linkages and ribose rings are low anti and 3′-*endo* of the N type, respectively, and that the backbone torsion angles α, β, γ, δ, ε and ζ (see the legend to Table 2 for definition) lie in the g^-, t, g^+, g^+, t and g^- ranges, respectively (with the exception of γ for U_1, where free rotation about the C4′–C5′ bond occurs), typical of an *A*-RNA type structure. The rise per base of ~ 3 Å for the U_1pU_2 step is comparable to that in *A*-RNA 11 (2·8 Å; Arnott *et al.*, 1973), while that of ~ 4 Å for the U_2pC step is significantly larger. The rotation per base for both steps is 40 to 45°, which is 7 to 12° larger than that found in *A*-RNA 11 (32·7°; Arnott *et al.*, 1973) and the stacking of the three bases is almost perfect. These differences can be accomplished by relatively small changes in the backbone

TABLE 2

Parameters describing the structure of UpUpC bound to tRNA^{Phe} in solution determined from model building based on the interproton distances given in Table 1

A. *Glycosidic bond and main-chain torsion angles*

	χ(°)	α(°)	β(°)	γ(°)	δ(°)	ε(°)	ζ(°)	Sugar pucker
Bound UpUpC								
U_1	−160(low anti)			[a]	90(g^+)	−150(t)	−80(g^-)	C3′-*endo*
U_2	−160(low anti)	−30(g^-)	160(t)	50(g^+)	80(g^+)	−140(t)	−90(g^-)	C3′-*endo*
C	−140(low anti)	−20(g^-)	160(t)	60(g^+)	90(g^+)			C3′-*endo*
A-RNA 11[b]	−164(low anti)	−60(g^-)	180(t)	48(g^+)	83(g^+)	−140(t)	−85(g^-)	C3′-*endo*

B. *Other structural parameters*

	Rise per base (Å)	Rotation per base (°)	Helical sense
Bound UpUpC			Right
UpU step	~3	40–45	
UpC step	~4	40–45	
A-RNA 11[b]	2·8	32·7	Right

The nomenclature and definition of all angles is that given by Dickerson & Drew (1981). The main-chain conformation angles are defined as P–$O5'\overset{\alpha}{}$–$C5'\overset{\beta}{}$–$C4'\overset{\gamma}{}$–$C3'\overset{\delta}{}$–$O3'\overset{\varepsilon}{}$–$P\overset{\zeta}{}$, with zero at the *cis* position and positive angles by clockwise rotation of the further pair of atoms. The glycosidic bond torsion angles (χ) are similarly defined: for pyrimidines O1′–$C1'\overset{\chi}{}$–N9–C2. The error in the estimation of the individual χ and δ angles is $\sim \pm 10°$. The values of the other main-chain conformation angles can be defined only within a range of $\sim \pm 20°$.

[a] In the case of U_1, there is free rotation about the C4′–C5′ bond.

[b] From the fibre diffraction data of Arnott *et al* (1973).

torsion angles. The largest changes are in the angles α(P–O5′) and β(O5′–C5′), which have mean values of $-25°$ and 160°, respectively, in the case of bound UpUpC compared to values of $-60°$ and 180°, respectively, in the case of *A*-RNA 11 (see Table 2). It is also interesting to note that that the structure of the anticodon triplet $G_{m34}A_{35}A_{36}$ in the monoclinic crystal form of $tRNA^{Phe}$ has a number of similar features to that found in UpUpC, in particular a large rotation per residue ($\sim 42°$), a low value of the angle β ($\sim 150°$), and almost perfect overlap of the bases (Jack *et al.*, 1976; Hingerty *et al.*, 1978).

(c) *Functional implications of the structure of bound UpUpC*

It has been known for a long time that the codon–anticodon complex is more stable than that found for two equivalent complementary ribotrinucleoside diphosphates and for complexes of ribotrinucleoside diphosphates bound to other complementary regions of tRNA (Jaskunas *et al.*, 1968; Borer *et al.*, 1974; Eisenger *et al.*, 1971; Freier & Tinoco, 1975; Grosjean *et al.*, 1976). The explanation for these observations may be found in the special structure of UpUpC bound to $tRNA^{Phe}$. The almost perfect base stacking and tight helical conformation (i.e. the large rotation angle per residue) of bound UpUpC imply complementary structural features for the bases of the anticodon triplet in the codon–anticodon complex. Given that the stability of oligonucleotide double helices depends to a large extent on the degree of base stacking, it is clear that the present structure would be expected to be significantly more stable than that of an equivalent double-helical RNA trimer with a conventional *A*-RNA structure.

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