Eur. J. Biochem. *145*, 359–364 (1984)

Yeast $tRNA^{Asp}$: codon and wobble codon-anticodon interactions

A transferred nuclear Overhauser enhancement study

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(Received July 11, 1984) – EJB 84 0754

The conformations of the ribotrinucleoside bisphosphates GpApC and GpApU, the codon and wobble codon for aspartic acid respectively, bound to yeast $tRNA^{Asp}$ in solution, have been examined by means of time-dependent transferred nuclear Overhauser enhancement measurements to determine distances between bound ligand protons. The conformations of the two bound ribotrinucleoside bisphosphates are shown to be very similar with an overall root-mean-square difference in interproton distances of 0.03 nm. The ribose conformations of all the residues are 3′-*endo*; the glycosidic bond torsion angles of the A and C residues of GpApC and of the A and U residues of GpApU are in the low *anti* range. These features are typical of an A-RNA type structure. In contrast, the G residue of both GpApC and GpApU exists as a mixture of *syn* and *anti* conformations. The overall conformation of the two bound ribotrinucleoside bisphosphates is also similar to A-RNA and the stability of the complexes is enhanced by extensive base-base stacking interactions. In addition, it is shown that the binding of the codon GpApC to $tRNA^{Asp}$ induces self-association into a multicomplex system consisting of four GpApC-$tRNA^{Asp}$ complexes, whereas the wobble codon GpApU fails to induce any observable self-association.

In a recent paper we presented a transferred nuclear Overhauser enhancement (TRNOE) study on the interaction of the triplet codon r(UpUpC) with yeast $tRNA^{Phe}$ [1]. From the TRNOE measurements intranucleotide and internucleotide interproton distances between protons of bound UpUpC were determined and used to solve the structure of bound UpUpC by model building. It was shown that the structure of bound UpUpC was similar to that of conventional A-RNA 11 but small differences in backbone torsion angles resulted in a special structure with a larger rotation per residue and almost perfect stacking of the bases. These two structural features which are complementary to those found in the anticodon triplet of the monoclinic crystal form of $tRNA^{Phe}$ [2, 3] have a functional significance in that they can account in part for the known greater stability of the codon-anticodon complex relative to an equivalent double-helical RNA trimer with a conventional A-RNA structure [4–8]. In the present paper we have extended the TRNOE studies to the complexes of the codon GpApC and the wobble codon GpApU with yeast $tRNA^{Asp}$ in order to obtain further insight into the structural aspects of codon-anticodon interactions.

EXPERIMENTAL PROCEDURE

The ribotrinucleoside bisphosphates GpApC and GpApU were chemically synthesized using the bifunctional phosphorylating reagent *o*-chlorophenyl-*O*,*O*-bis(1-benzotriazolyl) phosphate [9]. The exocyclic amine groups of guanosine, adenosine and cytidine were protected as benzoyl, benzoyl and anisoyl amides respectively. The 2′-hydroxyl functions were protected as tetrahydropyranyl ethers except for the 3′-terminal residues which contained 2′,3′-dibenzoyl-protected ribose moieties. The 5′-terminal hydroxyl was in each case protected as the 4,4′-dimethoxytrityl derivative. The fully protected products GpApC and GpApU were deprotected in three consecutive steps essentially as described elsewhere [9, 10] but without intermediate product purification. The fully deprotected products were isolated on columns (2 × 25 cm) of Sephadex A-25 using a linear gradient of 0.02–0.4 M of triethylammonium bicarbonate pH 7.5. The products were analyzed as described previously [11].

$tRNA^{Asp}$ was isolated from crude unfractionated bakers' yeast tRNA by chromatography on benzoylated DEAE-cellulose followed by isolation from Sephadex A-25; a final purification step was achieved by chromatography on a column (21 × 250 mm) of ODS-Hypersil which had been coated with trioctylmethylammonium chloride [12].

The samples for ^{1}H-NMR were freeze-dried extensively from 99.6% D_2O and finally dissolved in 99.96% D_2O buffer containing 144 mM KCl, 7.2 mM $MgCl_2$, 14.4 mM potassium phosphate pH* 7.0 (meter reading uncorrected for the isotope effect on the glass electrode) and 0.07 mM sodium NaEDTA. The concentrations of ribotrinucleoside bisphosphate and $tRNA^{Asp}$ used in the TRNOE experiments were 4.2 mM and 0.38 mM respectively, corresponding to a ratio of free to bound ligand of 10. All glassware was heated to 200°C for 4 h before use to inactivate possible traces of ribonuclease.

^{1}H-NMR spectra were recorded at 500 MHz on a Bruker AM500 spectrometer. The resonances of the free ribotrinucleoside bisphosphates were assigned by means of two-dimensional homonuclear *J*-correlated spectroscopy [13, 14]. The time-dependent TRNOEs were observed by directly

Abbreviations. NOE, nuclear Overhauser enhancement; TRNOE, transferred nuclear Overhauser enhancement; GpApC, guanylyl-(3′-5′)adenylyl(3′-5′)cytidine; GpApU, guanylyl-(3′-5′)adenylyl(3′-5′)-uridine.

collecting the difference free induction decay by interleaving eight transients after saturation for a set time of a given resonance, with eight transients of off-resonance irradiation (applied for the same length of time), negating the memory between eight transient cycles. The 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 an interpulse relaxation delay of 3 s. The irradiation power used was sufficient to be in the high power limit so that saturation was effectively instantaneous whilst selectivity was preserved so that only a single resonance at a time was saturated [15]. 208 transients were recorded for each difference spectrum and prior to Fourier transformation the difference free induction decays were multiplied by an exponential equivalent to a line broadening of 2 Hz. Chemical shifts are expressed relative to 4,4-dimethylsilapentane-1-sulphonate. All measurements were carried out at 5°C.

Model building was carried out manually using Nicholson skeletal models at a scale of 0.1 nm to 1 cm.

RESULTS AND DISCUSSION

Time-dependent TRNOE measurements

The nuclear Overhauser enhancement (NOE) is a method of both demonstrating the proximity of protons in space and determining their separation [16–22]. The TRNOE involves the extension of NOE measurements to exchanging systems, making use of chemical exchange to transfer magnetic information concerning cross-relaxation between bound ligand protons from the bound state to the free state [23, 24]. In this manner negative TRNOEs arising from cross-relaxation between bound ligand protons in a ligand-macromolecule complex (for which $\omega\tau_c \gg 1$, where ω is the Larmor frequency and τ_c the correlation time of the complex) can be easily observed on the readily detectable free or averaged ligand resonances following irradiation of other ligand resonances (free, averaged or bound) [23–29].

The theory of the time-dependent TRNOE for an exchanging system containing multiple spins as applied to the conformational analysis of ligands bound to macromolecules has been described in detail by Clore and Gronenborn [24] and will therefore only be briefly summarized here. In the case of the interaction of GpApC and GpApU with yeast $tRNA^{Asp}$, chemical exchange is fast on the chemical shift scale so that only a single set of exchange-broadened averaged ligand resonances is observed. In addition no NOEs could be observed between any pair of proton resonances at irradiation times of less than 1 s for the free ribotrinucleoside bisphosphates in the absence of $tRNA^{Asp}$. (This is as expected as $\omega\tau_c$ has a value close to 1 for molecules the size of the ribotrinucleoside bisphosphates such that the cross-relaxation rates and hence the steady-state NOEs have values very close to zero.) Under these conditions, the initial slope of the time development of the negative TRNOE, $N_i(j)$, observed on the averaged ligand resonance i following irradiation of the averaged ligand resonance j is simply given by $-(1-a)\sigma_{ij}^{BB}$ where a is the mole fraction of free ligand and σ_{ij}^{BB} the cross-relaxation rate between the bound ligand protons i_B and j_B. The cross-relaxation rate σ_{ij}^{BB} is proportional to $(r_{ij}^{BB})^{-6}$ where r_{ij}^{BB} is the distance between the bound ligand protons i_B and j_B. Thus distance ratios and distances, if one distance is known, can be obtained from the equation $r_{ij}^{BB}/r_{kl}^{BB} = (\sigma_{kl}^{BB}/\sigma_{ij}^{BB})^{1/6}$, providing the correlation time of the i-j and k-l interproton vectors are the same. A further consequence of the dependence of σ_{ij}^{BB} on r_{ij}^{BB} is that the value of σ_{ij}^{BB} decreases rapidly as r_{ij}^{BB} increases becoming negligible for $r_{ij}^{BB} \gtrsim 0.4$ nm. As a result a lag phase is observed in the time development of the TRNOE when $r_{ij}^{BB} \gtrsim 0.4$ nm.

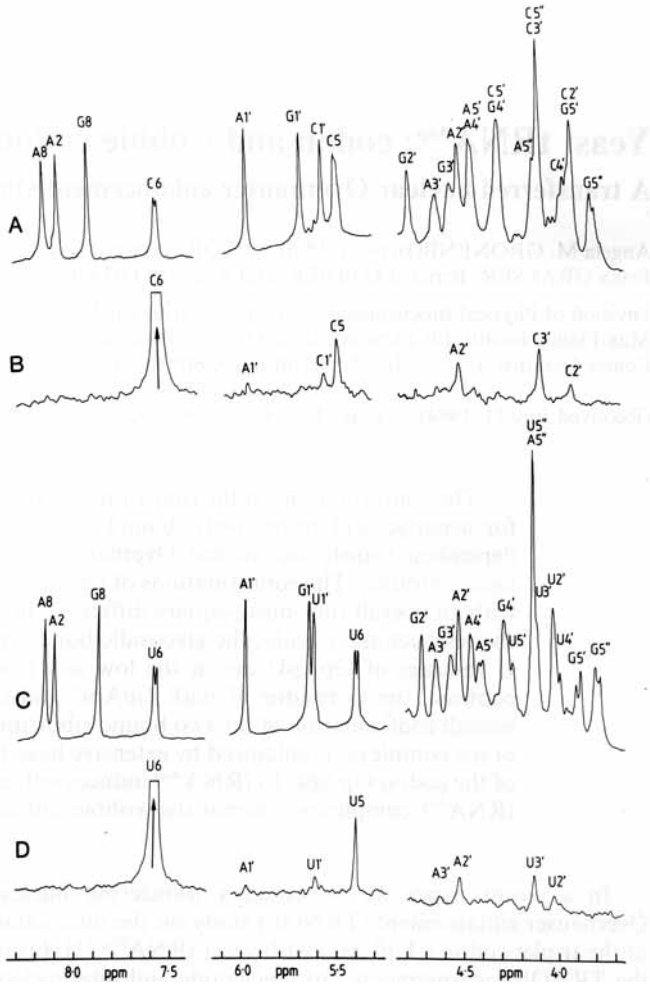

Fig. 1. *500-MHz ^{1}H-NMR spectra of 4.2 mM GpApC (A, B) and 4.2 mM GpApU (C, D) in the presence of 0.38 mM yeast $tRNA^{Asp}$ corresponding to a ratio of free to bound ribotrinucleoside bisphosphate of 10.* (A) Reference spectrum of GpApC in the presence of $tRNA^{Asp}$ together with resonance assignments; (B) TRNOE difference spectrum following pre-saturation of the averaged C(H6) resonance for 0.1 s. (C) Reference spectrum of GpApU in the presence of $tRNA^{Asp}$ together with resonance assignments: (D) TRNOE difference spectrum following pre-saturation of the averaged U(H6) resonance for 0.2 s. Experimental conditions: 4.2 mM ribotrinucleoside bisphosphate and 0.38 mM $tRNA^{Asp}$ in 99.96% D_2O containing 144 mM KCl, 7.2 mM $MgCl_2$, 14.4 mM potassium phosphate pH* 7.0 and 0.07 mM EDTA; temperature, 5°C

Fig. 1 shows the spectra of GpApC and GpApU in the presence of $tRNA^{Asp}$ (with a ratio of free to bound ligand of 10) together with the TRNOE difference spectra obtained on irradiation of the averaged C(H6) and U(H6) resonances. The corresponding time course of the TRNOEs are shown in Fig. 2. In both cases direct intranucleotide NOEs are observed on the H5 base and H1′, H2′ and H3′ sugar resonances (Fig. 2A and C), and direct internucleotide NOEs on the H1′ and H2′ resonances of the adjacent A residue (Fig. 2B and D). In addition, an indirect internucleotide is observed on the A(H3′) resonance as manifested by a lag phase in the time

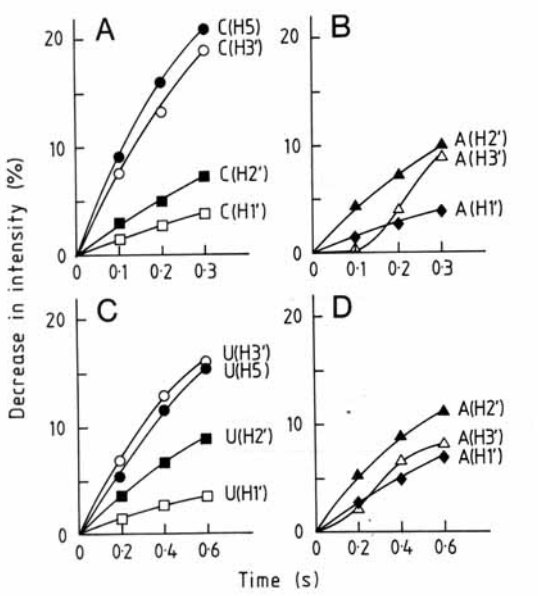

Fig. 2. *Time dependence of the TRNOEs observed following irradiation of the averaged C(H6) resonance of GpApC (A, B) and of the averaged U(H6) resonance of GpApU (C, D) in the presence of yeast tRNA^{Asp} with a ratio of free to bound ligand of 10.* The experimental conditions are as in the legend to Fig. 1

development of the TRNOE (Fig. 2B and D). Also note the more rapid time development of the TRNOEs for GpApC relative to those for GpApU, the significance of which is discussed in the following section.

The cross-relaxation rates between pairs of bound ribotrinucleoside bisphosphate protons determined from the initial slope of the time-dependent TRNOEs are summarized in Table 1 together with the $(<r^{-6}>)^{-1/6}$ mean interproton distances calculated from them using the distance (0.246 nm) and cross-relaxation rates between the H5 and H6 protons of the C (in the case of GpApC) and U (in the case of GpApU) bases as an internal reference.

Codon-induced self-association of tRNA^{Asp}

The correlation times τ_c of the ribotrinucleoside-bisphosphate–tRNA^{Asp} complexes can be obtained directly from the cross-relaxation rate and distance (0.246 nm) between the bound H5 and H6 protons of the C and U bases of GpApC and GpApU respectively using the equation:

$$\sigma_{ij}^{BB} = \frac{\gamma^4\hbar^2}{10\, r_{ij}^{BB6}}\left(\tau_c - \frac{6\tau_c}{1+4\omega^2\tau_c^2}\right)$$

where γ and $\hbar$ have their usual meanings [30, 31]. In this manner we obtain correlation times of ≈ 40 ns and ≈ 10 ns for the GpApC-tRNA^{Asp} and GpApU-tRNA^{Asp} complexes respectively. On the basis of the Stokes-Einstein equation, these values correspond to complexes of $M_r \approx 80000$ and ≈ 20000 respectively. We therefore conclude that the binding of the triplet codon GpApC to tRNA^{Asp} induces self-association into a multicomplex system consisting of four GpApC-tRNA^{Asp} complexes. In contrast, the wobble codon GpApU fails to induce any observable self-association.

The phenomenon of codon-induced self-association of tRNA^{Asp} is not unique to tRNA^{Asp} and has been observed for other tRNAs, in particular yeast tRNA^{Phe}, *Escherichia coli* tRNA^{Phe}, *E. coli* tRNA^{Lys} and *E. coli* tRNA^{fMet} [1, 32–34]. Sedimentation studies of this phenomenon carried out at low tRNA concentrations (< 30 μM) revealed only the presence of dimer formation [32–34]. However, the extent of self-association would be expected to depend on the concentration of codon-tRNA complexes so that the larger aggregates observed by the TRNOE measurements reported here on the GpApC-tRNA^{Asp} complex and previously on the UpUpC-tRNA^{Phe} complex [1] can easily be accounted for by the high concentrations ($\approx 0.4-0.5$ mM) of tRNA employed. Under the conditions used for the TRNOE experiments it is also interesting to note that in the case of yeast tRNA^{Phe}, codon-induced self-association is more effective than in the case of tRNA^{Asp}, as a multi-complex system consisting of 9 ± 2 UpUpC-tRNA^{Phe} complexes is formed [1]. This observation is correlated with the observation from sedimentation studies that the wobble codon UpUpU also induces self-association of yeast tRNA^{Phe}, although less effectively than UpUpC [33], whereas the wobble codon GpApU fails to induce any observable self-association of tRNA^{Asp}.

Conformations of GpApC and GpApU bound to tRNA^{Asp}

The structures of the ribotrinucleoside bisphosphates bound to tRNA^{Asp} were determined by model building on the basis of the intranucleotide and internucleotide interproton distance data (Table 1) using the principles described previously [35, 36]. From a comparison of the distance data (Table 2) it is clear that the conformations of the two ribotrinucleoside bisphosphates are very similar with an overall root-mean-square difference of 0.03 nm.

The intranucleotide interproton distance data for the A and C residues of GpApC and the A and U residues of GpApU are consistent with a single structure with a low *anti* conformation about the glycosidic bond and a 3′-*endo* sugar pucker as in A RNA. The values of the glycosidic (χ) and C4′-C3′ (δ) bond torsion angles for these residues are given in Table 3.

In the case of the G residue of both ribotrinucleoside bisphosphates, the sugar pucker conformations are also 3′-*endo*. However, the $(<r^{-6}>)^{-1/6}$ mean intranucleotide sugar-base interproton distances are not consistent with a single glycosidic bond conformation but with a *syn/anti* mixture consisting of approximately equal proportions of the two conformers. This *syn/anti* mixture is also reflected by the observation of internucleotide NOEs from the H8 proton of the G residue to both the H8 and H2 protons of the A residue arising from the *anti* and *syn* conformations of the G residue respectively.

The helical twist and rise can be estimated from the internucleotide interproton distances. Considering the ApC and ApU steps of GpApC and GpApU respectively, we find a right-handed helical twist of $\approx 25°$ and a helical rise of ≈ 0.3 nm. In the case of both the ApC and ApU steps the six-membered ring of the adenine base is almost perfectly stacked over the cytosine ring, thereby maximizing the contribution of the base-base stacking interaction to the overall interaction energy.

The situation in the case of the GpA step for both ribotrinucleoside bisphosphates is somewhat more complicated. Even taking account of the *syn/anti* mixture for the G residue, the internucleotide distances do not appear to be compatible with a single position of the ribose ring of the G residue relative to the A residue. Consequently, the helical rise and twist for the GpA step cannot be ascertained.

Table 1. *Corss-relaxation rates for GpApC and GpApU bound to tRNA^{Asp} determined from time-dependent TRNOE measurements together with the ($<r^{-6}>$)$^{-1/6}$ mean interproton distances calculated from them*

The experimental conditions are as in the legend to Fig. 1. The relative errors in the values of the cross-relaxation rates are $\lesssim 15\%$. The $(<r^{-6}>)^{-1/6}$ mean interproton distances are calculated using the distance (0.246 nm) and cross-relaxation rates between the H5 and H6 protons of the C (in the case of GpApC) and U (in the case of GpApU) bases as an internal reference. Assuming an error of ± 0.005 nm 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 $\lesssim 0.02$ nm. An internal quality control on the NMR distance determinations is provided by the other fixed internal distance, namely $r_{H5'-H5''}$ which has an idealized value of 0.18 nm and experimental values in the 0.18–0.19-nm range (with the exception of the G residue of GpApC; see footnote a)

(A) *Intranucleotide*

Distance	GpApC						GpApU					
	G_1		A_2		C_3		G_1		A_2		U_3	
	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}
Sugar-sugar:	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm
H1′-H2′	6.0	0.27	6.3	0.27	5.0	0.28	2.1	0.26	1.2	0.28	1.2	0.28
H1′-H4′	2.7	0.31	2.7	0.31	1.1	0.36	0.8	0.30	0.3	0.35		
H2′-H3′	6.8	0.26	6.9	0.26	8.9	0.25	1.6	0.27	1.8	0.26	2.3	0.25
H3′-H4′	6.0	0.27	6.8	0.26			0.9	0.29	0.8	0.30		
H3′-H5″	5.8	0.27					0.5	0.32	0.8	0.30		
H4′-H5″	7.8	0.26					2.5	0.25	3.5	0.23		
H5′-H5″	29[a]	—[a]	49	0.19			13	0.18	15	0.18	12	0.19
Sugar-base:												
H1′-H8/H6	3.5	0.29[b]	1.8	0.33	1.5	0.34	1.7	0.26[b]	0.3	0.35	0.5	0.31
H2′-H8/H6	3.8	0.29[b]	(lag)		2.9	0.30	0.8	0.30[b]	0.5	0.32	1.4	0.28
H3′-H8/H6	2.9	0.30[b]	3.4	0.29	8.7	0.25	0.7	0.31[b]	0.5	0.32	3.5	0.23
Base-base:												
H5-H6					10	0.25					2.6	0.25

(B) *Internucleotide*

5′-Nucleotide–3′-nucleotide	GpApC				GpApU			
	GpA		ApC		GpA		ApU	
	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}	σ_{ij}^{BB}	r_{ij}^{BB}
	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm	s^{-1}	nm
H1′-H8/H6	(lag)		1.5	0.34	0.8	0.30	1.3	0.28
H2′-H8/H6	7.0	0.26	4.2	0.29	1.5	0.27	2.7	0.25
H3′-H8/H6	2.9	0.30	(lag)		(lag)		(lag)	
H2′-H5			(lag)				0.6	0.31
H3′-H5			(lag)				0.5	0.32
H2-H1′			2.3	0.31			1.0	0.29
H8/H6-H8/H6	0.9	0.37	(lag)		0.7	0.30	(lag)	
H2′-H1′	1.3	0.35	(lag)		0.25	0.36	0.25	0.36
H8-H2	0.5	0.41[b]			0.5	0.32[b]		

[a] The correlation time calculated for the H5′-H5″ vector of the G residue of GpApC using the standard distance and measured cross-relaxation rate is 20 ns compared to a value of ≈ 40 ns calculated from the distance and cross-relaxation rate between the H5 and H6 protons of the C base. This can be accounted for by rapid internal motion about the C4′-C5′ bond: for the two-state jump model the amplitude of this internal motion would be $\approx \pm 25°$, whereas for the limited internal diffusion model it would be $\approx \pm 50°$ [43].

[b] These distances involving the G residue of both ribotrinucleoside bisphosphate are not compatible with a single conformation of the G residue and are indicative of a *syn/anti* mixture about the glycosidic bond of the G residue (see text for discussion)

CONCLUDING REMARKS

The findings reported in this paper on the interaction of the codon GpApC and wobble codon GpApU with tRNA^{Asp} and in the previous paper [1] on the interaction of the codon UpUpC with tRNA^{Phe} suggest certain common features of codon and wobble codon-anticodon interactions. The overall conformations of bound UpUpC and of the ApC and ApU moieties of bound GpApC and GpApU are of the A type. These structures, however, are modified from that of classical A RNA in such a way as to maximise base-base stacking interactions. This is achieved in part by an alteration in the helical twist: for the purine(5′)-pyrimidine(3′) step in GpApC and GpApU the helical twist ($\approx 25°$) is reduced whereas for

Table 2. *Root-mean-square (RMS) difference in the interproton distances of GpApC and GpApU bound to tRNA^{Asp}*

Distance	No. of distances	RMS difference
		nm
Intranucleotide	21	0.02
Internucleotide	7	0.05
Overall	28	0.03

Table 3. *Glycosidic (χ) and C4′-C3′ (δ) bond torsion angles for GpApC and GpApU bound to tRNA^{Asp} determined by model building on the basis of the intranucleotide interproton distances given in Table 1*
χ and δ are defined as follows: χ_{pur} = O1′−C1′$\frac{\chi}{}$N9−C4, χ_{pyr} = O1′−C1′$\frac{\chi}{}$N1−C2, and δ = C5′−C4′$\frac{\delta}{}$C3′−O3′, with zero at the *cis* position and positive angles by clockwise rotation of the further pair of atoms. The error in the estimation of the individual χ and δ angles is $\approx \pm 10°$

Nucleotide	Residue	χ	δ
GpApC		degrees	
	G	(*syn*/*anti*)	90
	A	−180	80
	C	−140	90
GpApU			
	G	(*syn*/*anti*)	90
	A	−150	90
	U	−140	90

the pyrimidine-pyrimidine steps in UpUpC the helical twist ($\approx 45°$ [1]) is increased relative to that of A RNA (33° [37]). From the functional viewpoint this has the consequence of stabilizing the codon and wobble codon-anticodon interactions relative to equivalent double-helical RNA trimers. Moreover it ensures that the energetic cost of employing the wobble codon rather than the codon is minimal. These structural features are also reflected to some extent in the monoclinic crystal form of tRNA^{Phe} [2, 3] and the orthorhombic crystal form of tRNA^{Asp} [38−40]; in both cases stacking interactions involving the anticodon triplet and the adjacent 3′-residues within the anticodon loop are increased relative to those in A-RNA 11. Interestingly in this respect, dimeric association of tRNA^{Asp} in the orthorhombic crystal form occurs by a direct anticodon-anticodon interaction involving complementary hydrogen-bonded base-pairing of the outer bases of the GUC anticodon triplet with the two anticodon triplets arranged in a helical conformation stabilized by stacking of the modified base m^{1}G^{37} on both sides [39]. (It should be noted that anticodon-anticodon interaction of tRNA^{Asp} also occurs in solution, albeit weakly, $K \approx 10^4\ M^{-1}$ [41], but would not affect the present results owing to the large excess of codon and wobble codon ribotrinucleotide bisphosphates over tRNA^{Asp} employed.)

However, in addition to these similarities, both bound GpApC and GpApU possess a structural feature that is quite distinct from that of bound UpUpC, namely the existence of a *syn*/*anti* conformational mixture of the G residue. The explanation of this finding may lie in alternative base-stacking configurations as deduced from model building. In the *anti* conformation there is intrastrand stacking of the G and A bases; in contrast, in the *syn* conformation, although intrastrand stacking can no longer occur, interstrand stacking of the G residue of the ribotrinucleoside bisphosphate with the m^{1}G^{37} residue of the anticodon loop can potentially take place. In this manner the loss in the *syn* conformation of the three hydrogen bonds arising from G(codon) · C^{34} (anticodon) base pairing may be compensated for by a more effective base-stacking configuration.

A further feature to emerge is that whereas codons induce self-association of the codon-tRNA complex, the wobble codon either fails to do so as in the case of tRNA^{Asp} or does so with considerably reduced efficiency as in the case of tRNA^{Phe} [33]. This implies a distinct conformational change in some part of the tRNA molecule upon codon binding. In this respect ^{1}H-NMR studies on a pentadecamer comprising the anticodon loop and stem of yeast tRNA^{Phe} have shown that the 3′-stacked conformation of the loop remains unchanged upon codon binding [42]. This suggests that a long-range conformational change affecting other parts of the tRNA molecule may be responsible for self-association of codon-tRNA complexes. The functional significance of codon-induced self-association of the codon-tRNA complex is unknown. However, it is clear that one possible role could involve the stabilization of the translation complex with two tRNA molecules after recognition of a contiguous codon, thereby supporting successful transfer of the growing peptide chain [33], particularly if association also occurs between heterologous codon-tRNA complexes. If this is indeed the case, then the choice of codon or wobble codon could serve an intrinsic regulatory function in governing the efficiency of translation of a given mRNA.

This work was supported by the Medical Research Council (GMC and AMG), the Lister Institute of Preventive Medicine (GMC) and the Max-Planck-Gesellschaft (LWM). GMC is a Lister Institute Research Fellow. tRNA^{Asp} was isolated during a stay in Göttingen by BL supported by a short-term EMBO fellowship. The NMR spectra were recorded on the AM500 spectrometer of the Medical Research Council Biomedical NMR Centre at the National Institute for Medical Research.

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