Base-Analog-Induced Aminoacylation of an RNA Helix by a tRNA Synthetase

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At least eight examples of sequence-specific aminoacylation of RNA oligonucleotides have been reported, including aminoacylations with alanine, aspartic acid, glycine, histidine, isoleucine, methionine, serine, and valine.[1] Although aminoacylation efficiency is reduced compared to that obtained with the full tRNA, high specificity is observed. The sequence-specific aminoacylations of RNA oligonucleotides can be viewed as an operational RNA code for amino acids whereby sequences/structures in tRNA acceptor stems, including specific 2′-hydroxyl groups,[2] correspond to specific amino acids.[3] The substrates in these instances are based on tRNA acceptor stems and consist of helical segments with as few as four base pairs joined to a common single-stranded $NCCA_{OH}$ at the 3′-end. A single G3:U70 acceptor stem base pair is a major determinant for aminoacylation with alanine.[4,5] The unpaired, exocyclic 2-amino group of G3 positioned in the minor groove by this wobble pair is required for aminoacylation.[6] Here, we report the first examples of base-analog-induced aminoacylation reactions and show that charging depends significantly on isolated atoms *per se*, regardless of the bases to which they are attached.

We took advantage of chemical synthesis[7] to incorporate 2-aminoadenosine[8] (2-AA) and isocytidine[9] (isoC) into an RNA duplex substrate[10] at the position of the G3:U70 base pair. A 2-AA:isoC pair is isosteric with G:U and should place the 2-amino group of 2-AA in the same location in the minor groove as that of a G:U base pair (Figure 1).[11] The resulting hybridized duplexes recreated the first nine base pairs of the acceptor-TψC helix and 3′-terminal $ACCA_{OH}$ of *Escherichia coli* $tRNA^{Ala}$ (Figure 1).

The 2-AA:isoC-containing duplex was efficiently aminoacylated by *E. coli* alanyl-tRNA synthetase (Figure 2a),[12] with an activity (k_{cat}/K_M) within a factor of 3 of that for the "wild-type" substrate which has the G3:U70 base pair. Neither the G3:C70, I3:U70, 2-AP3:U70 (2-AP: 2-aminopurine) nor A3:U70 substrates are charged with alanine.[13] These substrates either lack the unpaired 2-amino group (I3:U70 and A3:U70) or present it in a Watson−Crick hydrogen bond (G3:C70 and 2-AP3:U70) (Figure 1).

With these considerations in mind, we synthesized a 2-AA3:U70 duplex. The expectation was that, because the 2-amino group of 2-AA is hydrogen bonded in a Watson−Crick conformation (Figure 1), this substrate should not be aminoacylated. This expectation was confirmed (Figure 2a).

We also synthesized a duplex containing a G3:isoC70 base pair. In this instance, alternative pairings can be envisioned, depending on the tautomeric state of the isocytidine ring (Figure 1). Paired with the imine−oxo tautomer of isoC, the 2-amino group of G is unpaired in the minor groove and could serve as a signal for aminoacylation. Although the imine−oxo tautomer of 1-methylisocytosine predominates in N_2 matrices,[14] the predominant tautomer of isocytosine is known to depend on environment,[15−18] and in particular, the proportion of imine−oxo tautomer in aqueous solution is unknown.

As might be expected if some imine−oxo tautomer did form, we observed a significant albeit reduced (approximately 10-fold relative to the "wild-type" duplex) initial rate of aminoacylation of the G3:isoC70 duplex (Figure 2b).[19] As an alternative explanation for this activity, aminoacylation of the

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(3) Schimmel, P.; Giegé, R.; Moras, D.; Yokoyama, S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8763−8768.
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(6) Musier-Forsyth, K.; Usman, N.; Scaringe, S.; Doudna, J.; Green, R.; Schimmel, P. *Science* **1991**, *253*, 784−786.
(7) Oligonucleotides were synthesized on a either a Pharmacia Gene Assembler Plus or an Applied Biosystems 394 automated synthesizer.
(8) 2-Aminoadenosine was prepared from guanosine (Fox, J. J.; Wempen, I.; Hampton, A.; Doerr, I. L. *J. Am. Chem. Soc.* **1958**, *80*, 1669−1675). Following protection (Naito, T.; Ueno, K.; Ishitzawa, F. *Chem. Pharm. Bull.* **1964**, *12*, 951−954. Kikuchi, K.; Tamiyama, Y.; Marumoto, R. *Z. Naturforsch.* **1988**, *43b*, 623−630. Usman, N.; Ogilvie, K. K.; Jiang, M.-Y.; Cedergren, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845), the resulting 5′-*O*-dimethoxytrityl-2′-*O*-triisopropylsilyl-N^2,N^6-diacetyl-2-aminoadenosine derivative was converted to the 3′-*O*-(β-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite following standard procedures (Nielsen, J.; Dahl, O. *Nucleic Acids Res.* **1987**, *15*, 3626. Milecki, J.; Denbek, I.; Antkowiak, W. Z.; Gdaniec, Z.; Mielewezyk, S.; Adamiak, R. W. *Nucleosides Nucleotides* **1989**, *8*, 463−474).
(9) Isocytidine was prepared via 2,5′-anhydrouridine (Brown, D. M.; Todd, A. R.; Varadaraian, S. *J. Chem. Soc. (London)* **1957**, 868−872. Shibuya, S.; Kuninata, A.; Yoshiuo, H. *Chem. Pharm. Bull.* **1974**, *22*, 719−721) and converted to 5′-*O*-dimethoxytrityl-2′-*O*-triisopropylsilyl-N^4-(*N*,*N*-dimethylaminomethylene)isocytidine (Zemlicka, J.; Holy, A. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3159−3168. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827. Usman, N.; Ogilvie, K. K.; Jiang, M.-Y.; Cedergren, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845). The corresponding 3′-*O*-(β-cyanoethyl-*N*,*N*-diisopropyl) phosphoramidite was then prepared as described for 2-AA.
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(12) Assays were carried out at room temperature in buffer containing 50 mM HEPES (pH 7.5), 100 μg/mL BSA, 20 mM KCl, 10 mM $MgCl_2$, 20 mM BME, 4 mM ATP, 22.4 μM [2,3-^{3}H]alanine (4.44×10^{-4} μCi/pmol). Preannealed RNAs (80 °C for 3 min, then cooled on ice for 20 min) were added to a final concentration of 2 μM (except that the RNA concentration was 4.5 mM in the U3:G70 assay). Reactions were initiated by addition of *E. coli* alanyl-tRNA synthetase (2 μM active site), and aliquots (8 μL) were removed at the indicated times and analyzed as described previously.[10]
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(19) *N*-Acyl protected isocytidine derivatives have been reported to undergo hydrolytic deamination when subject to base deprotection conditions. (See: Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, *32*, 10489−10496. See also ref 11.) Protection of the amino group as a Schiff base (*N*,*N*-dimethylaminomethylene) affords greater stability, however. In model studies, no deamination product (uridine, <1%) was detected by TLC upon treatment of *N*,*N*-dimethylaminomethylene-isocytidine using our deprotection conditions (3:1 NH_3 (aqueous, 32%):EtOH at 55 °C for 24 h). In contrast, N-isobutyrylisocytidine shows ~5% deamination using this procedure. Thus, we feel that it is unlikely that the observed aminoacylation of the G:isoC construct arises from U contamination as a result of deamination.

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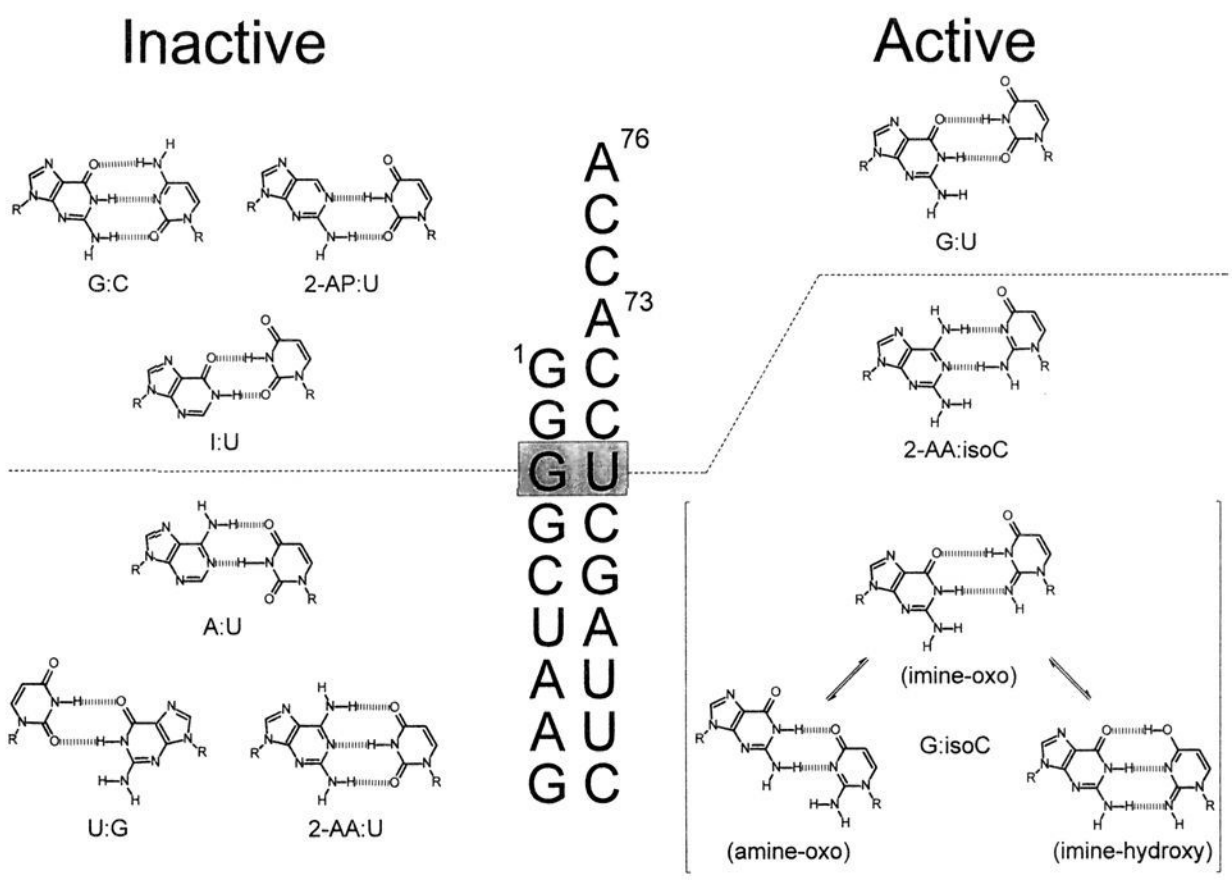

Figure 1. RNA duplex substrate used for aminoacylation experiments (center) and illustration of base pairs investigated at the 3:70 position (shaded base pair) for aminoacylation with alanine. Nucleotides of the duplex substrate are numbered in accordance with their positions in *E. coli* $tRNA^{Ala}$ (GGC and UGC isoacceptors). Inactive and active variants in this and previous work are shown on the left and right of the duplex, respectively. The constructs unique to this study are shown below the dashed line on each side and include A:U, U:G, 2-aminoadenosine:isocytidine (2-AA:isoC), 2-AA:U, and G:isoC.

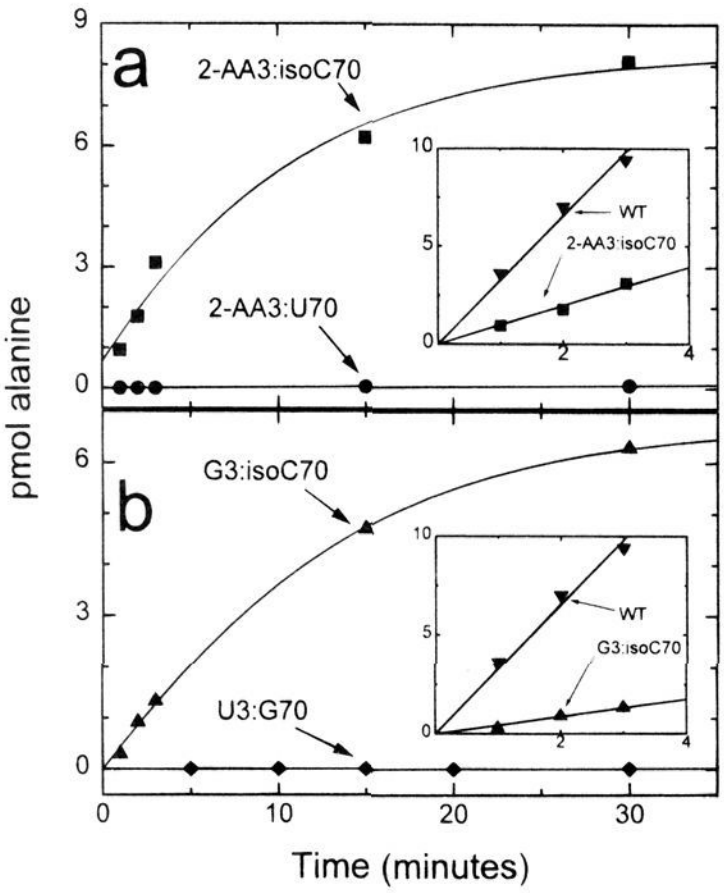

Figure 2. Aminoacylation of RNA duplexes with substitutions of the 3:70 position. (a) Comparison of substrates with 2-AA:isoC and 2-AA:U substitutions. (Inset) Initial rates of aminoacylation for the 2-AA:isoC-containing and wild-type (G:U, labeled WT) substrates. (b) Comparison of substrates with G:isoC and U:G substitutions. (Inset) Initial rates of aminoacylation for the G:isoC-containing and wild-type (G:U, labeled WT) substrates.

G3:isoC70 duplex could arise from the unpaired amino group donated from the opposite strand by the amine−oxo tautomer of isoC (Figure 1). Because an unpaired 2-amino group from the opposite strand is also created by transversion of G:U to U:G, we constructed a U3:G70-containing duplex. This substrate was devoid of aminoacylation activity (Figure 2b). Thus, although the G3:U70 and U3:G70 base pairs each present an unpaired 2-amino group to the minor groove, the enzyme is able to distinguish the difference in angle at which the nitrogen with its pendant hydrogens protrudes into the minor groove.

The results presented here demonstrate that the active substrate for aminoacylation with alanine is not dependent on a specific base pair but rather on a specific atomic group presented by natural and non-natural base pairs. In comparing G3:U70 with 2-AA:isoC, for example, the 2-AA:isoC construct (Figure 1) places in the major groove the 6-amino group of 2-AA at the location of the 6-keto oxygen of G3 and places in the minor groove the 2-amino of isoC at the position of the 2-keto of U70 (Figure 1). Thus, the lowering of the transition state free energy by the unpaired amino group in the minor groove is achieved in the context of variations in the fine structure of the local minor and major grooves. It is noteworthy that minor groove recognition of the exocyclic 2-amino group of a G−U wobble base pair contributes substantially to RNA recognition of a group I intron splice site, thereby suggesting a parallel between RNA−RNA and RNA−protein recognition.[20]

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