J|A|C|S
COMMUNICATIONS

Published on Web 12/13/2003

# Recognition of G-1:C73 Atomic Groups by *Escherichia coli* Histidyl-tRNA Synthetase

Abbey E. Rosen and Karin Musier-Forsyth*

*Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455*

Received August 27, 2003; E-mail: musier@chem.umn.edu

Histidine tRNA is the only tRNA with an extra G-1 nucleotide at the 5′-end[1] opposite the so-called "discriminator" base, N73.[2] In eukarya, N73 is an adenine, creating a G-1:A73 mismatch, while in bacteria and archaea it is a cytosine, increasing the number of acceptor stem base pairs from seven to eight. Here, we use atomic group "mutagenesis" to identify functional groups in the unique G-1:C73 base pair of $tRNA^{His}$ that mediate efficient aminoacylation by class II *Escherichia coli* histidyl-tRNA synthetase (HisRS). Earlier studies have shown that G-1:C73 in *E. coli* $tRNA^{His}$ is the major recognition element for HisRS.[3−5] Microhelices of eight base pairs that mimic the acceptor stem of *E. coli* $tRNA^{His}$ are aminoacylated by HisRS, and incorporation of G-1:C73 into $microhelix^{Ala}$ and $microhelix^{Gly}$ confers histidylation upon these substrates.[4,5] More recently, the 5′-phosphate of G-1 was identified as a critical recognition element.[6]

In this work, we used chemically synthesized $microhelix^{His}$ variants to probe specific atomic groups of the unique G-1:C73 base pair. Backbone mutations confirmed the importance of the 5′-monophosphate of G-1 in the microhelix system but showed that the 2′-hydroxyl groups of G-1 and C73 are dispensable for aminoacylation (Table 1). In particular, deletion of the 5′-monophosphate (HO-G-1:C73) resulted in a 510-fold decrease in aminoacylation catalytic efficiency, which is in good agreement with results in the full-length $tRNA^{His}$ system.[6] This large effect suggests that the 5′-monophosphate contributes 3.6 kcal/mol to transition-state stabilization.

Previous studies of $tRNA^{His}$ G-1:C73 recognition were restricted, in part, due to the limitations of in vitro RNA transcription reactions.[7] Moreover, G-1 variants cannot be studied in vivo due to processing of mutant −1 bases by RNase P.[8] We prepared and tested all six individual standard base substitutions in the microhelix system. While the A-1:C73 and C-1:C73 substitutions reduced activity by 230- and 310-fold, respectively, U-1:C73 substitution resulted in only a 46-fold decrease (Table 1). Both the A-1 and C-1 substitutions result in an exocyclic amine group in the major groove at the −1 position, while U-1 maintains the keto oxygen in this position, similar to wild-type G-1 (Figure 1). At the 73 position, standard base substitutions of U and G resulted in inactive mutants (i.e. >1200-fold decrease in $k_{cat}/K_m$). For the G-1:A73 variant, which maintains the major groove amine of C73, a $k_{cat}/K_m$ of 0.011 (96-fold decrease) was measured (Table 1). Taken together, these results suggest HisRS recognizes the major groove of G-1:C73.

Base analogue substitutions were next used to further probe atomic groups in the major and minor grooves of G-1. Substitution of inosine (I-1:C73), which lacks the minor groove amine, and 2′-deoxy-7-deazaguanine (7DAG-1:C73), which has a carbon in place of the major groove N7 nitrogen, resulted in only modest changes in aminoacylation activity, +1.8- and −2.4-fold, respectively (Table 1). However, removal of the keto oxygen at position 6 of G-1 by substitution of 2-aminopurine (2AP-1:C73) resulted in a $microhelix^{His}$ variant that was 200-fold less active than wild-type (Table 1). This significant decrease in activity could be due to the loss of the major groove functionality and/or the predicted shift in the conformation of the base pair from a Watson−Crick to a wobble configuration (Figure 1). The latter effect is expected to slightly alter the position of the critical 5′-monophosphate.

***Table 1.*** Aminoacylation Efficiency of $Microhelix^{His}$ Variants

| −1:73 base pair[a] | $k_{cat}/K_m$ (relative)[b] | fold decrease[c] | $-\Delta\Delta G^{\ddagger}$ (kcal/mol)[d] |
|---|---|---|---|
| G:C (wild-type) | 1 | 1 | 0 |
| Backbone Substitutions | | | |
| G:dC | 0.84 | 1.2 | 0.1 |
| dG:C | 1.1 | 0.91 | −0.065 |
| HO-G:C | 0.0021 | 510 | 3.6 |
| C73 Substitutions | | | |
| G:A | 0.011 | 96 | 2.7 |
| G:U | 0 | >1200 | >4.2 |
| G:G | 0 | >1200 | >4.2 |
| G-1 Substitutions | | | |
| A:C | 0.0044 | 230 | 3.2 |
| C:C | 0.0032 | 310 | 3.4 |
| U:C | 0.022 | 46 | 2.3 |
| 7DAG:C | 0.43 | 2.4 | 0.50 |
| I:C | 1.8 | 0.60 | −0.34 |
| 2AP:C | 0.0050 | 200 | 3.1 |
| 2AA:C | 0.0018 | 580 | 3.8 |
| Pur:C | 0.0016 | 590 | 3.7 |
| Base-Pair Substitutions[e] | | | |
| isodG:isoC | 0 | >1200 | >4.2 |
| ΔG-1 Substitutions[f] | | | |
| ΔG:C | 0.00086 | 1200 | 4.2 |
| Abasic Substitutions[g] | | | |
| Ab:C | 0.019 | 48 | 2.3 |

[a] All microhelices were synthesized on an Expedite 8909 RNA synthesizer (Applied Biosystems) and have a 5′-monophosphate with the exception of the HO-G:C variant. Nonstandard bases are abbreviated as follows: 7DAG: 7-deaza-2′-deoxyguanine, I: inosine, 2AP: 2-aminopurine, 2AA: 2-aminoadenine, Pur: purine, isoC: isocytidine, isodG: 2′-deoxyisoguanine, Ab: 2′-deoxyabasic. [b] Values reported are the average of at least three determinations with average standard deviations of ±26%. [c] Fold decrease in $k_{cat}/K_m$ is given relative to wild-type $microhelix^{His}$. [d] $-\Delta\Delta G^{\ddagger}$ is defined as $RT \ln[(k_{cat}/K_m)^{variant}/(k_{cat}/K_m)^{wild-type}]$, where $R = 1.98272$ cal/mol·K and $T = 298$ K. [e] Other base-pair substitutions tested (A:U, U:A, C:G, 2AA:U, Pur:U) were inactive. [f] All other ΔG-1 substitutions tested (ΔG-1:A73, ΔG-1:G73, ΔG-1:U73) were inactive. [g] All other abasic substitutions tested (G:Ab, Ab:A, Ab:G, Ab:U, Ab:isoC, Ab:isodG) were inactive.

To try to distinguish between its role as a positioning element for the 5′-phosphate group versus its direct role in recognition, we replaced G-1 with a 2′-deoxyabasic (Ab) residue, which deleted the guanine but preserved the sugar and 5′-monophosphate (Figure 1). This substitution resulted in a 48-fold decrease in aminoacylation efficiency ($-\Delta\Delta G^{\ddagger} = 2.3$ kcal/mol, Table 1). Thus, removal of the base alone is ∼10-fold less deleterious than deletion of the 5′-phosphate. As expected, deletion of the entire nucleotide (ΔG-1:C73) resulted in a 1200-fold decrease in aminoacylation activity (Table 1). Taken together, these results suggest that although a major


10.1021/ja0381609 CCC: $27.50 

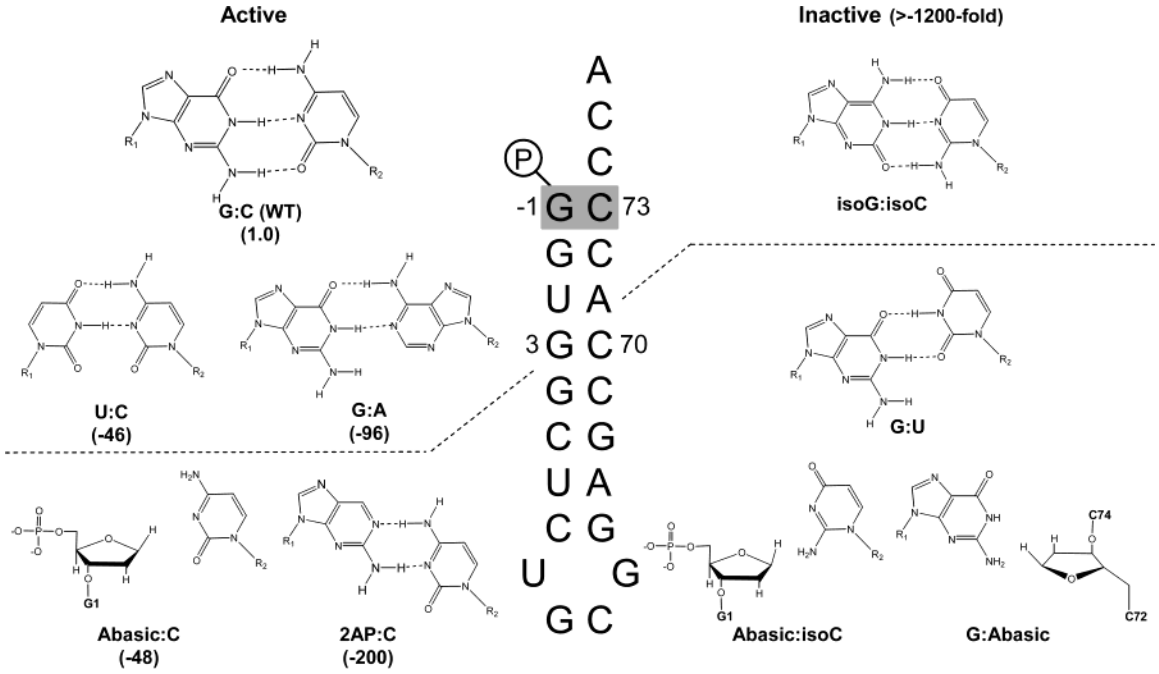


***Figure 1.*** Microhelix substrate used for aminoacylation experiments (center) and some of the base pairs substituted at the −1:73 position (shaded). Active and inactive variants in this work are shown on the left and right of the microhelix, respectively, with proposed Watson−Crick-like base pairs above the dashed line and non-Watson−Crick base pairs (i.e. wobble pairs or pairings with abasic residues) shown beneath. Numbers in parentheses are fold decrease in $k_{cat}/K_m$ relative to wild-type.

role of the extra nucleotide is to position the critical 5′-phosphate as previously suggested,[6] major groove G-1 functional groups also play a role in HisRS recognition. Furthermore, the 48-fold decrease observed upon substitution of Ab-1:C73, suggests that at least part of the 200-fold decrease upon 2AP-1 substitution may be attributed to a conformational change that likely affects the positioning of the 5′-phosphate.

Deletion of the cytosine base at position 73 (G-1:Ab73) resulted in an inactive variant (Figure 1). This result is in accord with previously published in vivo results showing C73 is critical to *E. coli* histidine identity.[8] Comparison of Ab-1:C73 (−48-fold) to Ab-1:isoC73 (>−1200-fold, Figure 1) further supports the importance of C73 functional groups. The latter substitution switches the positions of the major and minor groove atomic groups of C73. Activity was also not recovered when the isodG-1:isoC73 microhelix$^{His}$ variant was tested (Figure 1), which implies efficient aminoacylation does not require simply a purine:pyrimidine Watson−Crick base −1:73 pair. The substitution of Ab-1:U73 also resulted in an inactive substrate, which suggests that the C73 major groove exocyclic amine is a site of recognition by HisRS and serves as a hydrogen bond donor. Our results cannot exclude the possibility that the 2-keto oxygen is also a site of recognition.

Throughout evolution, the extra G-1 nucleotide is conserved.[1] This nucleotide serves as a blocking element to prevent aminoacylation by noncognate synthetases.[5] Our data support the conclusion that another role for G-1 is to correctly position the critical 5′-monophosphate as well as to provide a major groove 6-keto oxygen for recognition.

In contrast to G-1, the discriminator base of tRNA$^{His}$ is not universally conserved. It had been previously suggested that the lack of strict conservation through evolution might indicate that atomic group recognition by HisRS occurs at the N73 position.[8] Indeed, specific deletion of the cytosine base at position 73 increases the free energy of activation by >4.2 kcal/mol. Thus, substitutions at this position are generally more detrimental than substitutions at G-1. Taken together, our results support the conclusion that *E. coli* HisRS recognizes the major groove exocyclic amine of C73.

In class II synthetases, major groove interactions at the top of the tRNA acceptor stem are generally observed,[9,10] and our results support a similar trend at the G-1:C73 base pair of *E. coli* tRNA$^{His}$. The major groove amine of C73 and the 6-keto oxygen of G-1 most likely participate in hydrogen bonding with HisRS, while the 5′-monophosphate provides necessary electrostatic interactions with the positive residues of the active site.

**Acknowledgment.** We are grateful to Drs. Christopher Francklyn and Susan Connelly for valuable discussions and critical reading of the manuscript. This work supported by N.I.H. Grant GM49928 and N.I.H. Predoctoral Training Grant T32-GM08700.

**Supporting Information Available:** Details on all materials and methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Sprinzl, M.; Horn, C.; Brown, M.; Ioudovitch, A.; Steinberg, S. *Nucleic Acids Res.* **1998**, *26*, 148−153.
(2) Crothers, D. M.; Seno, T.; Söll, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3063−3067.
(3) Himeno, H.; Hasegawa, T.; Ueda, T.; Watanabe, K.; Miura, K.-I.; Shimizu, M. *Nucleic Acids Res.* **1989**, *17*, 7855−7863.
(4) Francklyn, C.; Schimmel, P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8655−8659.
(5) Francklyn, C.; Shi, J.-P.; Schimmel, P. *Science* **1992**, *255*, 1121−1125.
(6) Fromant, M.; Plateau, P.; Blanquet, S. *Biochemistry* **2000**, *39*, 4062−4067.
(7) Milligan, J. F.; Uhlenbeck, O. C. *Methods Enzymol.* **1989**, *180*, 51−62.
(8) Yan, W.; Francklyn, C. *J. Biol. Chem.* **1994**, *269*, 1−7.
(9) Ruff, M.; Krishnaswamy, S.; Boeglin, M.; Poterszman, A.; Mitschler, A.; Podjarny, A.; Rees, B.; Thierry, J. C.; Moras, D. *Science* **1991**, *252*, 1682−1689.
(10) Cusack, S.; Yaremchuk, A.; Tukalo, M. *EMBO J.* **1996**, *15*, 2834−2842.

JA0381609