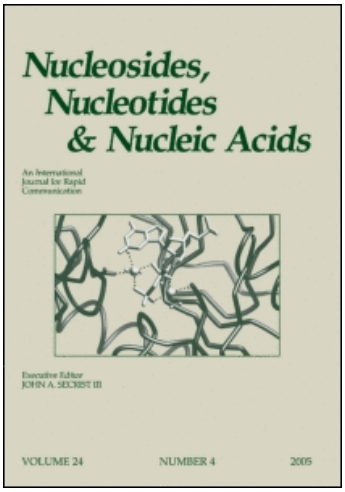

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Thermodynamics of Site-Specific Variant tRNA^{Ala} Acceptor Stem Microhairpins

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THERMODYNAMICS OF SITE-SPECIFIC VARIANT $tRNA^{Ala}$ ACCEPTOR STEM MICROHAIRPINS

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ABSTRACT. Thermal denaturation studies were carried out on a set of site-specific variants of a 22mer RNA hairpin comprising the aminoacyl acceptor stem sequence of *E. coli* $tRNA^{Ala}$. The pairing thermodynamics were calculated from the melting profiles.

The major determinant for the alanylation of *E. coli* $tRNA^{Ala}$ by its cognate alanine-tRNA-synthetase (AlaRS) is a G·U wobble base pair at position 3·70 in the acceptor helix of the tRNA.[1] *In vitro* alanylation assays using AlaRS and various synthetic $tRNA^{Ala}$ acceptor helix sequence variants lead to the conclusion that the unpaired 2-amino group of guanine in the minor groove at position 3 is a prerequisite for efficient alanylation and therefore must be directly recognised by the enzyme.[2]

In contrast, *in vivo* aminoacylation assays revealed that several other mutant tRNAs bearing mispairs other than G3·U70 – C3·A70, G3·A70, C3·C70, C3·U70, U3·U70, and A3·C70 – are alanylated with efficiencies of 38 to 80 % of the wild type tRNA.[3] Since there are no obvious common direct recognition elements in the above mispairs, these results suggest that the unpaired 2-amino group of G3 may not be directly recognised. Moreover, a high-resolution NMR structure of a G3·U70-containing acceptor stem microhairpin showed that the guanine base of G3 occupies the same position within the helix as guanine in a Watson-Crick G·C pair [4] and, consequently, that the unpaired 2-amino group of G3 does not protrude into the minor groove where it could be specifically recognised by the enzyme. The structural and aminoacylation data point to a dynamic component of $tRNA^{Ala}$ recognition and provide the basis for the present investigation.

Since the introduction of heat can simulate the overcoming of the free energy of activation by the enzyme during tRNA molecule binding, we seeked to compare the pairing thermodynamics of the acceptor stem of $tRNA^{Ala}$ with its function *in vivo*. To this end, we undertook a UV absorbance-detected thermal denaturing study of synthetic oligoribonucleotides that correspond to the above acceptor stem variants of $tRNA^{Ala}$ (Figure). We calculated the thermodynamics of base pairing from the melting profiles. The analysis discloses a unique melting behavior and enthalpy/entropy compensation of the G3· U70 variant, but also suggests how the absence of the wild type mispair may be overcome by the enzyme.

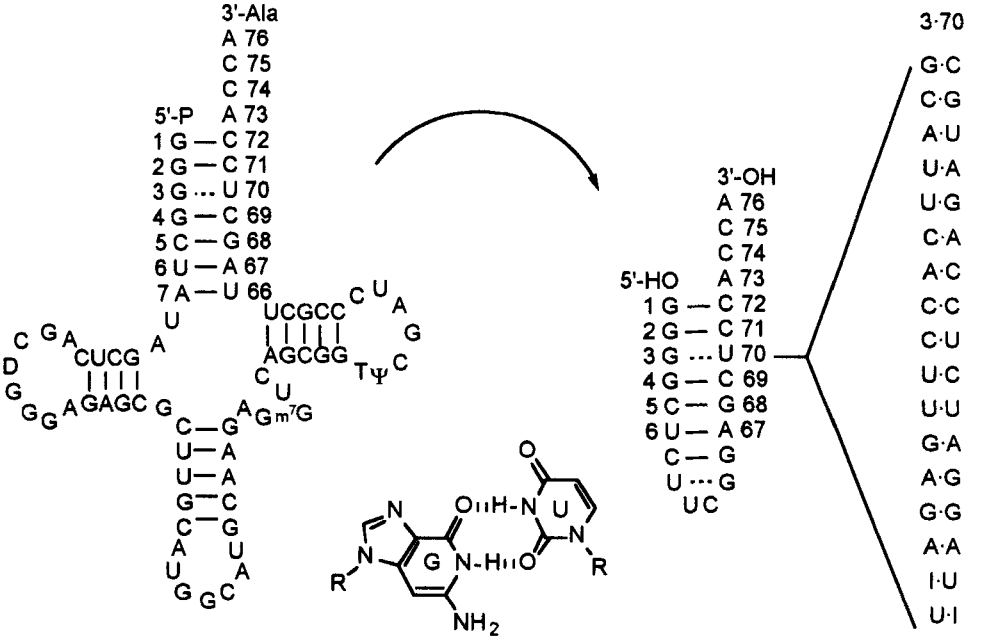

FIGURE *Left*: cloverleaf structure of wild type *E. coli* Ala-$tRNA^{Ala}$(GGC). *Right*: wild type acceptor stem microhairpin and 3·70 base pair variants (I: inosine). *Bottom*: G·U wobble pair.

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