

Sequence of tRNA^{Asp} from *Thermus thermophilus* HB8

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The tRNA^{Asp} from *Thermus thermophilus* HB8 has been isolated at high purity for sequencing using conventional purification steps; we report here its primary structure.

Methods Bulk tRNA was prepared from *T.thermophilus* HB8 grown at 70 C; after phenol extraction, the tRNA^{Asp} was enriched by FPLC on a TSK butyl (Toyopearl 650S) column and further purified by two successive HPLCs on Phenomenex W-POREX 5 C4, and DEAE-VYDAC oligonucleotide columns. Purification was achieved by denaturing polyacrylamide gel electrophoresis. The tRNA^{Asp} was identified by aminoacylation using cloned aspartyl-tRNA synthetase from *T.thermophilus* (1). The nucleotide sequence was determined by single hit hydrolysis followed by postlabelling as already described (2).

Results Only one aspartic acid accepting peak was found despite two post-transcriptional heterogeneities in the primary structure of tRNA^{Asp} (Figure 1): indeed the position 8 (S⁴U) and 54 (S²T) are incompletely modified (only little S⁴U could be found in position 8, whereas 50% of the molecules carried T instead of S²T in position 54). The tRNA^{Asp} from *T.thermophilus* is 77% identical to that from *E.coli* (3) but some differences are worth mentioning: the tRNA from *T.thermophilus* has 3 additional GC pairs as compared to that from *E.coli* (17 and 14 respectively). The expected higher thermostability of that tRNA as compared to the *E.coli* one could result from i) Higher GC pairs content and ii) Special post-transcriptional modifications i.e. Gm in position 18, S²T in 54 and m¹A in 58 as previously suggested for tRNA^{Ile} from *T.thermophilus* (4). In addition no modifications were found in the anticodon loop as compared to *E.coli* tRNA^{Asp} which contains Q in the wobble position of the anticodon and M²A in position 37 next to the anticodon.

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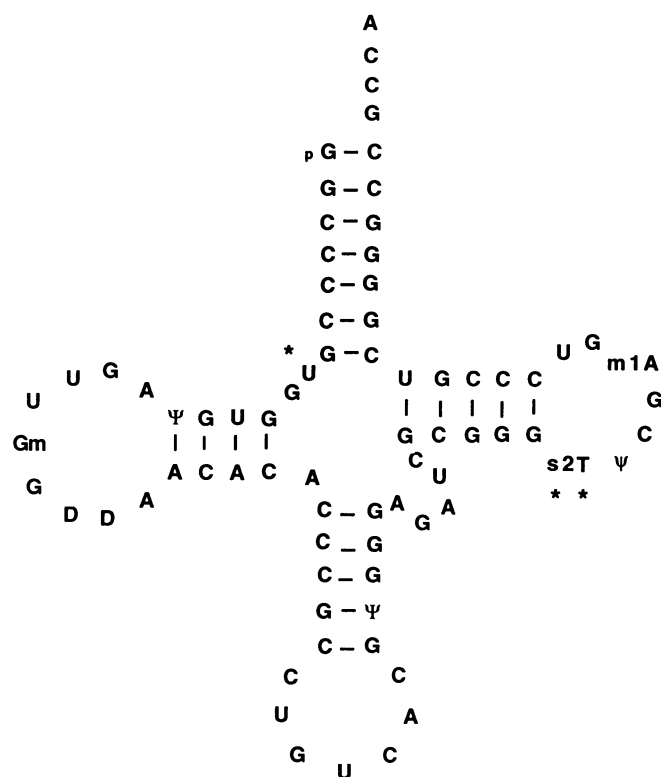


Figure 1. Cloverleaf drawing of *T.thermophilus* tRNA^{Asp}. * Some molecules display the presence of S⁴U. ** Approximately 50% of the tRNAs contain T instead of S²T.