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Single particle cryo-EM is an emerging technique to determine structures of proteins and RNA-protein complexes at near-atomic resolution. RNA molecules have not been a focus of cryo-EM studies, potentially due to their intrinsically high heterogeneity. The Tetrahymena group I intron was the first ribozyme discovered, and has since become a model system for studying RNA catalysis and the structure-function relationship. Here we report the cryo-EM structure of a catalytically active 388-nt (125 kDa) Tetrahymena thermophila L-21 ScaI ribozyme at 3.1 Å resolution. The newly resolved peripheral regions form two coaxially stacked helices connected by two pseudoknots (PKs), wrapping around the highly conserved catalytic core (stem P3-P9), and rigidifying the core by RNA tertiary interactions between stems P2 and P4, and stems P9.1 and P7. In addition, the core structure is further stabilized by RNA tertiary interactions that have not been observed in previous crystal structures. Metal ions essential to this metalloenzyme have been unambiguously identified, most of which agree with previous crystal structures of Tetrahymena and other group I introns. The cryo-EM model including metal ions is validated by Q-score analysis. These results unveil a complete view with novel structural insights of this ancient RNA catalyst, and demonstrate the unique capability of cryo-EM in RNA structural studies.