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Folding and binding stability of the ε and θ subunits of DNA polymerase III. DANIEL SPENCER, Institute of Molecular Biophysics, Florida State University, AH YOUNG PARK, NICHOLAS E. DIXON, Research School of Chemistry, Australian National University, HUAN-XIANG ZHOU, Institute of Molecular Biophysics & Department of Physics, Florida State University — The ε subunit of DNA polymerase III is responsible for the proofreading and repair functions of the holoenzyme during DNA replication. The θ subunit binds to the ε subunit. This binding has been suggested to provide additional folding stability to the ε subunit. We have studied the folding stability and the binding affinity of the two subunits at 15 $^\circ$C. The midpoint of urea denaturation of the ε subunit was low, at 2.4 M urea, but the slope of the unfolding free energy versus urea was high (at 2.9 kcal/mol/M). The sensitivity to urea echoes the low thermal stability. In contrast, the midpoint of urea denaturation of the θ subunit was high, at 3.8 M urea, but the slope of the unfolding free energy versus urea was low (at 1.0 kcal/mol/M). Both proteins thus showed marginal stability with respect to denaturation. Their complex exhibited much greater resistance to denaturation, with a midpoint of urea denaturation at 4.3 M.

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