The *E. coli* HscA/HscB chaperone/cochaperone system facilitates iron-sulfur cluster transfer from holo-IscU to apo-ferredoxin in an ATP-dependent manner

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The iron sulfur cluster assembly operon (*isc*) in *E. coli* encodes two proteins proposed to function as scaffolds for preassembly of transient [FeS] clusters, IscU and IscA, and a specific hsp70-class chaperone/J-type cochaperone system consisting of HscA and HscB. IscU interacts specifically with HscA and HscB suggesting that the chaperones may play a role in assembly of FeS clusters on IscU or transfer of FeS clusters from IscU to acceptor apo-proteins. However, direct evidence of such a role has not been reported to date.

In this work, we studied cluster transfer from $IscU_2$-[2Fe2S] and $IscA_2$-[2Fe2S] complexes to apo-ferredoxin (Fd) in the presence/absence of the various components of the *isc*-encoded chaperone system. Cluster transfer was monitored by taking advantage of the intense visible-near UV circular dichroism (CD) signals of holo-Fd relative to the weaker CD features of holo-IscA and holo-IscU.

Addition of HscA and HscB together with Mg-ATP was found to increase significantly the rate of cluster transfer from holo-IscU to apo-Fd, and the observed increase showed a linear dependence on the added amount of HscA and HscB. Omission of either HscA, HscB, or ATP resulted in the absence of accelerating effects with respect to the "background" cluster transfer rate from holo-IscU to apo-Fd measured in the absence of the chaperone system. In contrast to the effects observed with IscU, addition of HscA, HscB, and Mg-ATP had no detectable effect on the rate of cluster transfer from holo-IscA to apo-Fd.

A detailed kinetic analysis of the accelerating effects of HscA and HscB on the rate of cluster transfer from holo-IscU is currently under way.

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