Multiple Turnover 2Fe2S Cluster Transfer Activity of IscA and IscU

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IscU/ISU and IscA/ISA (and the related NifU and SufA) have been proposed to serve as molecular scaffolds for pre-assembly of [FeS] clusters for the biogenesis of iron-sulfur proteins. This function has received support from several in vitro studies, which have demonstrated transfer of preformed scaffold-[FeS] complexes to apoprotein acceptors, but studies to date have yielded only single transfer events.

We developed an in vitro assay system based on chemical reconstitution procedures that allows for real-time monitoring of [FeS] cluster formation using circular dichroism (CD) spectroscopy. We used this to investigate de novo Fe-S cluster formation and transfer under conditions where the concentration of the apo-form of either scaffold protein was rate-determining.

By using nearly stoichiometric amounts of Li_2S and ferric/ferrous salts as the sources of cluster atoms, we took advantage of the intense visible CD signals in holoferredoxin (as compared to the relatively weak CD features in holoIscA/U) and of the relatively slow "background" rate of holoferredoxin formation in the absence of scaffold proteins, to record and analyze time courses of holoferredoxin formation in the presence of sub-stoichiometric concentrations of either apoIscU or apoIscA.

Under these conditions, both apoIscU and apoIscA from *E. coli* were capable of carrying out multiple cycles of [2Fe2S] cluster formation and transfer to *E. coli* apoferredoxin, suggesting that these scaffold proteins can act "catalytically".

Kinetic studies further showed that cluster transfer exhibits Michaelis-Menten behavior indicative of complex formation between holo-IscU/A with apo-ferredoxin and of a direct [FeS] cluster transfer mechanism. The catalytic activity of the two scaffold proteins was ostensibly different, IscU being by far more efficient than IscA both in terms of affinity towards the acceptor protein (K_m = 27 *vs* 210 μM) and of cluster transfer activity at saturating concentrations of the acceptor apoprotein (turnover number = 0.21 *vs* 0.029 min^{-1}).

Surprisingly, analysis of the dependence of the rate of cluster transfer on the ratio of scaffold protein to apoprotein acceptor revealed significantly enhanced transfer rates at ratios less than 0.2:1.0. This finding suggests that a participation of a transient, labile scaffold-[FeS] species formed during initial cluster assembly in the transfer process.

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