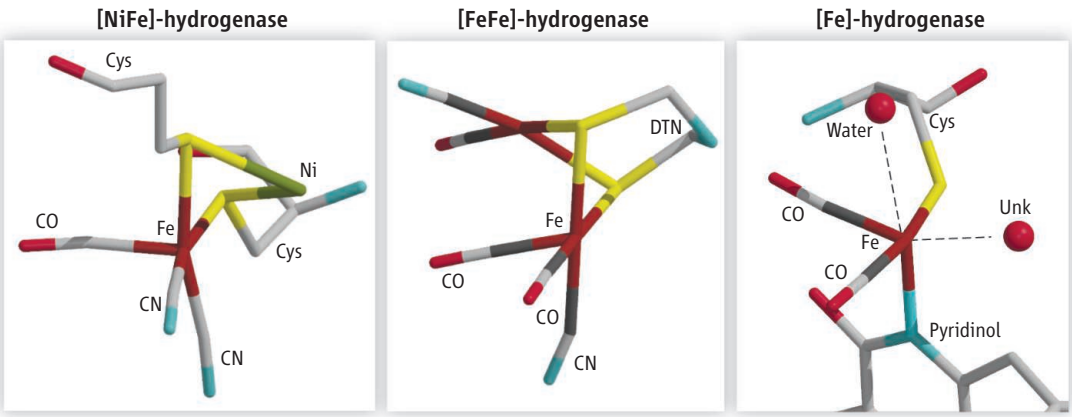

The active sites of the three classes of hydrogenase. The coordination centers of the equivalent iron centers are oriented to highlight their similarities. (**Left**) Fragment of the [NiFe]-hydrogenase active site (*2*). (**Center**) The binuclear [FeFe] subcluster of the [FeFe]-hydrogenase active site (*3*). DTN, di(thiomethyl)amine. (**Right**) Partial view of the active center of [Fe]-hydrogenase reported by Shima *et al.* (*1*). Unk, unknown ligand; this site appears to bind CN^-. The water molecule trans to the pyridinol ligand occupies the site thought to bind H_2.

hydrogenases, the presumed role of the second metal is to orchestrate, in part or in full, the two one-electron transfers that subsequently convert H^- to H^+. The [Fe]-hydrogenase is thus very important for our understanding of H_2 biocatalysis in general because it fulfills all the requirements for its activation and scission, the first stages of H_2 oxidation by the classic hydrogenases.

The three classes of active site share a $Fe(CO)_x(RS^-)$ unit (see the figure). The most notable feature of the [Fe]-hydrogenase (right panel) is that the coordination sphere lacks an intrinsic CN^- ligand (a stronger σ donor but weaker π acceptor than CO) and contains instead the N atom of a pyridinol ring attached to a guanyl mononucleotide. The two intrinsic CO ligands are arranged in a cis configuration. The remaining coordination sphere has some unexplained features. The site occupied by an unknown ligand is most likely the target for extrinsic CN^-. It is unlikely that this site is the one used for binding H_2 because CN^- is a noncompetitive inhibitor. In addition, a spherical electron density peak at 2.7 Å from the iron atom is modeled as a water molecule occupying the sixth coordination site. The sixth site is postulated to bind the competitive inhibitor CO and the substrate, H_2. The active site of the [Fe]-hydrogenase can be removed and transferred between related enzymes but is highly sensitive to light. This latter feature complicated its characterization substantially.

A low-spin Fe(II) site with five permanent two-electron donor ligands is well suited for binding a sixth ligand such as H_2. As described by Kubas (*6*), binding and activation of H_2 at a transition metal involves a subtle interplay between σ donation (required for binding) and back-donation (required for H-H cleavage), and it is noteworthy that the pyridinol N atom occupies the position trans to the vacant site at which H_2 probably binds. 2-Pyridinol is a versatile ligand (*7*): It can exist in two different forms with different electronic characters (an enol form with sp^2-hybridized N and a keto form with sp^3-hybridized N), can be deprotonated (increasing its donor properties), and resembles carboxylate in being potentially bidentate; indeed, it is most often encountered as a chelating ligand bridge between two metal atoms. In the [Fe]-hydrogenase, it is bound as a unidentate ligand, and the planarity of the Fe-ring system shows that N is sp^2-hybridized.

Changes in ligand status (perhaps induced by binding methenyl-H_4MPT) could play a key role in balancing σ-donor and π-acceptor characteristics at different stages of the catalytic cycle.

The remarkable convergent evolution that resulted in the presence of a common active-site $Fe(CO)_x(RS^-)$ unit in all three classes of hydrogenases deserves a comment. The π-acceptor nature of CO (and hydrogen-bonded CN^-) stabilizes low-spin, low-valence Fe with catalytic and hydride-binding properties that are normally found in second- and third-row transition metals, most notably platinum. Presumably, such metals were not available during evolution, and nature found a solution involving the much more common Fe ion and the abundant ligands CO and CN^-.

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BIOCHEMISTRY

Cargo Load Reduction

Ineke Braakman and Mieko Otsu

A specific enzyme that reduces protein disulfide bonds is part of a complex that eliminates aggregated, misfolded proteins.

In eukaryotic cells, most newly synthesized secretory proteins are first translocated into the endoplasmic reticulum (ER) and transit through organelles that constitute a secretory pathway. However, a fraction of them never reach the desired native state. Instead, these proteins misfold in the ER and are retrotranslocated out of this organelle to the cytoplasm, where they are degraded by the ubiquitin-proteasome system (a process called ER-associated degradation). For efficient retrotranslocation, the disulfide bonds of misfolded proteins must be reduced, and on page 569 in this issue, Ushioda *et al.* (*1*) report that this reaction is catalyzed by ERdj5, the first dedicated reductase identified in the ER.

The most abundant ER oxidoreductase, protein disulfide isomerase (PDI), contains two thioredoxin-like domains Cys-X-X-Cys (CXXC, where X is another, but not any, amino acid), and can make (oxidize), break (reduce), and isomerize disulfide bonds, depending on reaction conditions (*2*). In principle, PDI can do the reductase job as well as ERdj5 (*3*).

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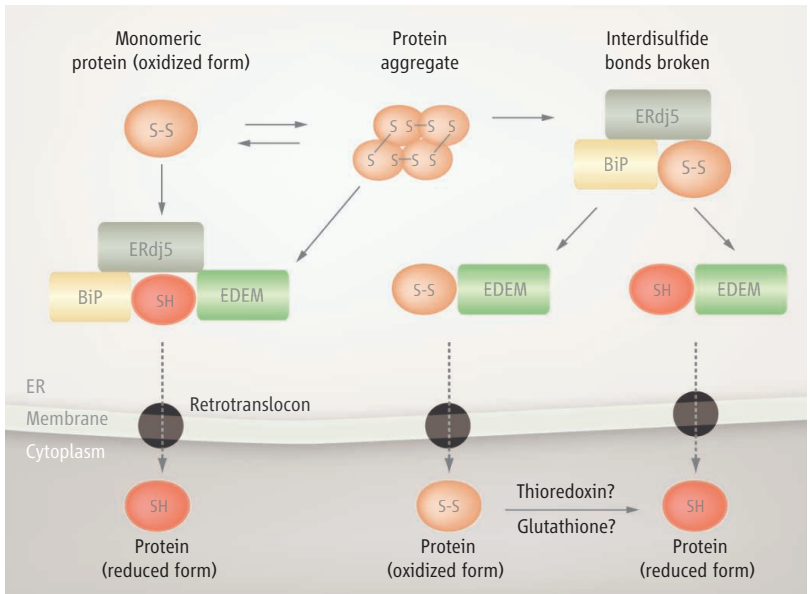
However, more than 20 additional ER-resident PDI family proteins with at least one CXXC motif have been found in mammalian cells (*4*), suggesting dedicated functionality compared to the broad activity of PDI. Ushioda *et al*. show that ERdj5 has neither oxidase nor isomerase activity in vitro, although it has four CXXC motifs (*5*, *6*). It also contains a J domain, which is found in proteins that assist heat shock protein 70 (Hsp70) in functioning as a chaperone in protein folding. This indicates that ERdj5 could function as a co-chaperone for BiP, an ER-resident protein that is a member of the Hsp70 chaperone family (*5*, *6*). Further specificity is generated by ERdj5 binding to the ER-degradation-enhancing α-mannosidase–like protein (EDEM), the lectin that receives misfolded glycoproteins from the chaperone calnexin, and directs them out of the ER to the cytoplasm (*7*, *8*). By coupling BiP and EDEM, ERdj5 may open the degradation path to misfolded proteins and aggregates (see the figure).



ER-associated degradation. Possible roles for the enzyme ERdj5 are shown. ERdj5 reduces interchain (and perhaps intrachain) disulfide bonds in misfolded and aggregated proteins in the ER, which prevents aggregation, helps dissolve aggregates, or both. ERdj5 associates with BiP and EDEM and may perform this activity as a ternary complex, directly targeting glycoproteins for retrotranslocation and degradation in the cytoplasm. Alternatively, ERdj5 and BiP first may act together on an aggregated substrate, delivering either fully reduced or oxidized monomers to the retrotranslocon. If proteins are retrotranslocated with disulfide bonds intact, both deglycosylation and reduction in the cytosol will be needed for efficient degradation.

Why is it important to reduce disulfide bonds at all when oxidized immunoglobulin kappa chain (*9*), bulky glycosylated proteins (*10*), and intact virus (simian virus 40) (*11*) can be ejected from the ER through a retrotranslocon (whose identity remains ambiguous)? It may be that instead of extending a protein chain, reduction releases proteins from thiol-mediated retention in the ER, or breaks apart protein aggregates with the combined action of BiP. Ushioda *et al*. find that ERdj5 decreases the amount of disulfide-linked aggregates of immunoglobulin J chain and dimers of α_1-antitrypsin. A disulfide-linked aggregate may be either too large to pass through retrotranslocons, or detained by a large number of chaperones, whereas individual monomeric molecules are likely optimal substrates for retrotranslocation.

Combined ERdj5 and BiP activities are not limited to glycoproteins; nonglycoproteins are substrates as well (*12*). In this case, EDEM is bypassed and BiP may directly target substrates to the retrotranslocation machinery (*9*). The combination of an oxidoreductase and chaperone in the ER is also not unique: ERp57 functions with the chaperones calnexin and calreticulin (*13*), and PDI harbors both functions. And although ERdj5 accelerates misfolded protein degradation, it is not crucial for this process because reduced expression of ERdj5 did not obliterate degradation. Any of the ER-resident PDI family proteins could function redundantly with ERdj5. On the other hand, the effects of the complete removal of ERdj5 expression are not known, and a small amount of this protein may be sufficient to stimulate degradation.

As well, reduction of disulfide bonds is not unique to protein degradation. Proper protein folding also involves constant reduction of nonnative disulfide bonds. Because ERdj5 catalyzes this process, a pool of ERdj5 should exist that is not bound to EDEM, which is underscored by its action on nonglycoprotein substrates.

A reductase must be kept in a reduced form and therefore needs an electron donor. In vitro, dithiothreitol or glutathione can fulfill this role, but in cells, electrons must be provided inside the ER. Analogy with the bacterial periplasmic space (where electron transport processes occur) suggests a role for cytosolic thioredoxin, whereas oxidases donate their electrons to proteins in an independent electron transport cascade. However, cytosolic electron donation requires a transmembrane domain–containing protein for this transport step. Instead, Ushioda *et al.* suggest ER-localized nicotinamide adenine dinucleotide phosphate as an alternative electron donor. A theme among oxidoreductases is the cooperation of two active sites within one protein, and the four active sites of ERdj5 in principle lend themselves to such a mechanism.

Although proteins with major roles in ER-associated degradation have been characterized, and several proteins have been put forward as the retrotranslocon—Sec61 (*14*), derlins (*15*–*17*), and lipid droplets (*18*)—it is fair to say that the retrotranslocation mechanism remains a black box. How proteins cross the ER membrane to the cytosol still is an open question. Another major question is why there are so many oxidoreductases in mammalian cells, given that PDI is sufficient to carry out all disulfide-reducing activity and can act on most substrates tested. Answers will likely come from studies that address tissue and substrate specificity, and—as exemplified by ERdj5's association with BiP and EDEM—from partnerships that direct activity toward a specific process.

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