A possible therapeutic target for Lou Gehrig’s disease
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In 1939, after playing 2,130 consecutive games at first base for the New York Yankees, Lou Gehrig missed a game. Two years later, at the age of 37, he was dead. Lou Gehrig’s disease, or amyotrophic lateral sclerosis (ALS), afflicts ~35,000 Americans. Similar to Alzheimer’s disease (AD) and Parkinson’s disease (PD), ALS is a late-onset neurodegenerative disease characterized by protein aggregates that colocalize with neuronal loss (1, 2). Unlike AD and PD, ALS progresses very rapidly, and most patients die within 5 years of diagnosis, often from asphyxia. There is no effective treatment. However, an article in this issue of the PNAS (3), taken together with a recent article from our laboratory (4), suggests a therapeutic target and offers hope that this situation could soon change. We imagine that the progression of ALS could be significantly slowed by a drug that would prevent aggregation of a ubiquitous enzyme.

In ~10% of cases, ALS is transmitted in an autosomal-dominant manner [familial ALS (FALS)] (5, 6). The most commonly mutated gene, accounting for ~20% of all FALS, encodes superoxide dismutase type 1 (SOD1), a dimeric metalloenzyme that is rich in β-sheet structure and contains copper- and zinc-binding sites, the former being critical for catalysis (Fig. 1). FALS has been linked to ~100 SOD1 mutations, which are scattered throughout the three-dimensional structure (7). The A4V mutation has been frequently studied because it produces a rapidly progressive disease characterized by protein aggregates under others (4, 19). A4V SOD1 has an increased propensity to aggregate (17), forming amyloid fibrils and amyloid pores under conditions (12, 18) and amyloid pores under others (4, 19). Hough et al. (3) describe the crystal structures of dimeric forms of A4V and another FALS mutant, I113T. Both proteins retain the same monomer fold and active-site geometry as WT but are twisted in a corkscrew fashion relative to each other, consistent with a destabilization of the interface [A4V did not produce this effect when placed in a non-WT background (C6A/C111A), suggesting that residues 6 and 111 interact with residue 4 (18)].

Based on previous studies, monomeric A4V (and partially unfolded species derived from it), the twisted A4V dimer, and the A4V aggregates, especially the amyloid pore (19, 20), were all candidate pathogens (see Fig. 2). All these species could be derived from a single pathway that starts with dimer dissociation. Alternatively, aggregation may not require dimer dissociation (but the two may be linked). To choose viable therapeutic targets, it is important to distinguish between these possibilities. We therefore produced a covalently linked variant of A4V by engineering an unstrained intersubunit disulfide bond across the A4V dimer interface (see Fig. 2) (4). The A4V/V148C protein formed a stable disulfide-linked dimer (A4V/V148C)2 that did not aggregate in vitro, suggesting that the monomer is an obligate intermediate along the aggregation pathway. Thus, a drug-like molecule that stabilizes the SOD1 dimer could inhibit the formation of all the potentially pathogenic species. Such a molecule could delay onset and slow the progression of FALS.

The general strategy of inhibiting potentially pathogenic aggregation by sta-
that is responsible for carrying l-thyroxine (T4) in plasma and cerebrospinal fluid. Two equivalents of T4 bind in symmetry-related sites in the central cavity of TTR; each T4 molecule makes contact with two TTR subunits. T4 binding stabilizes the TTR tetramer and slows the rate of tetramer dissociation, which is the rate-determining step of \textit{in vitro} TTR fibril formation (22). Several approved drugs bind to the TTR tetramer in an analogous manner as T4, inhibit TTR dissociation and aggregation (23, 24), and prevent aggregation-associated toxicity in cell culture (24). Compounds in this class will soon enter clinical trials for FAP (J. Kelly, personal communication).

Although designed for SOD1-linked FALS, this strategy may also be applicable to sporadic ALS, because pathogenesis in that case may be initiated by dissociation of WT dimer (accelerated by complex factors, such as abnormal metal metabolism or chaperone activity, rather than a mutation). Unfortunately, native protein stabilization may not be applicable to AD or PD, because Ap (AD) and α-synuclein (PD) are disordered targets, making drug binding entropically disfavored. Although curing ALS by this strategy is unlikely, the more likely outcome, converting ALS into a slow-progressing chronic disease similar to PD, would be a great benefit to patients and their families.

Amyloid polyneuropathy (FAP). FAP is linked to point mutations in the gene encoding the protein transthyretin (TTR) (21). TTR is a tetrameric protein that is responsible for carrying l-thyroxine (T4) in plasma and cerebrospinal fluid. Two equivalents of T4 bind in symmetry-related sites in the central cavity of TTR; each T4 molecule makes contact with two TTR subunits. T4 binding stabilizes the TTR tetramer and slows the rate of tetramer dissociation, which is the rate-determining step of \textit{in vitro} TTR fibril formation (22). Several approved drugs bind to the TTR tetramer in an analogous manner as T4, inhibit TTR dissociation and aggregation (23, 24), and prevent aggregation-associated toxicity in cell culture (24). Compounds in this class will soon enter clinical trials for FAP (J. Kelly, personal communication).

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