L55P Transthyretin Accelerates Subunit Exchange and Leads to Rapid Formation of Hybrid Tetramers

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Transthyretin is a tetrameric protein associated with the commonest form of systemic amyloid disease. Using isotopically labeled proteins and mass spectrometry, we compared subunit exchange in wild-type transthyretin with that of the variant associated with the most aggressive form of the disease, L55P. Wild-type subunit exchange occurs via monomers and dimers, whereas exchange via dimers is the dominant mechanism for the L55P variant. Because patients with the L55P mutation are heterozygous, expressing both proteins simultaneously, we also analyzed the subunit exchange reaction between wild-type and L55P tetramers. We found that hybrid tetramers containing two or three L55P subunits dominate in the early stages of the reaction. Surprisingly, we also found that, in the presence of L55P transthyretin, the rate of dissociation of wild-type transthyretin is increased. This implies interactions between the two proteins that accelerate the formation of hybrid tetramers, a result with important implications for transthyretin amyloidosis.

A number of models have been proposed to explain the mechanism of fibril formation for transthyretin. Although there is general consensus that dissociation of the tetramer at low pH (7) correlates with the formation of amyloid fibrils in vitro (8), it has not been established whether it is monomeric or dimeric species that are ultimately involved in fibril formation. Evidence for the role of monomeric transthyretin in amyloidogenesis comes from studies of a variant designed to be monomeric in solution (9). This variant was found to form fibrils >100 times faster than wild-type transthyretin under low pH conditions but to remain soluble at physiological pH. Partial denaturation of the monomer is also required to produce a conformation that is then capable of assembly to amyloid fibrils in vitro (9, 10).

An alternative model involves dissociation of the transthyretin tetramer into amyloidogenic dimers. Support for this hypothesis comes from investigations of a dimeric variant that is prone to amyloid formation (11) and another that forms cytotoxic spherical aggregates (12). Spin labeling experiments have demonstrated that many of the interactions found in the native transthyretin dimer are maintained in fibrils (13). The role of dimers is also suggested by the numerous x-ray studies of transthyretin and its disease-associated variants (14), and it has been hypothesized that the extended β-sheet structure, which comprises the β-sheet interface, is a starting point for amyloid fibril formation (15, 16).

Although it is not clear whether a universal mechanism for transthyretin fibril formation will emerge for the various transthyretin variants, the situation is further complicated in vivo, because individuals suffering from familial amyloidotic polyneuropathy are (in general) heterozygous, co-expressing both wild-type and variant proteins in the liver (17–19). However, the process of subunit exchange, which was first postulated more than 30 years ago (20), before the tetrameric structure of transthyretin was even realized, is of particular interest in the heterozygous case. Although most investigations have focused on homogeneous transthyretin tetramers, formation of hybrid tetramers of V30M or A25T and wild-type transthyretin have been reported from experiments employing a 16-amino-acid anionic tag (21–23). Ion exchange chromatographic separation demonstrated that the kinetics of subunit exchange in the V30M mutation are closely similar to those measured for the wild-type protein. Using a similar approach, a model for the subunit exchange process in the wild-type protein has recently been proposed (23). In this model, rate-limiting tetramer dissociation to four monomers is followed by rapid reassociation of the monomers to form mixed tetramers.

Here we investigated the most aggressive of the disease-associated transthyretin variants, that of L55P. We describe a novel mass spectrometry (MS) approach whereby the subunit composition of intact wild-type and L55P tetramers was monitored as a function of time by incubating solutions in which one set of homotetramers possessed only natural abundance isotopes and the other was uniformly labeled with

The abbreviation used is: MS, mass spectrometry.
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FIGURE 1. Structure of the wild-type transthyretin tetramer (A) and the same structure rotated by 90° around the y-axis (B). The hydrophobic and β-sheet interfaces are shown with dashed lines, with residues involved in forming the hydrophobic interface highlighted in red and the H- and F-strands, which form hydrogen bonds required to stabilize the β-sheet interface, highlighted in yellow. Residue Leu-55 is shown in green for one of the monomers. Adapted from 1ICT (40).

13C and 15N throughout. This methodology avoided the use of chemical modification and allowed us to resolve, in real time, the assembly of the five tetramers with unique masses that formed as a result of subunit exchange. A detailed analysis of the kinetics of this process revealed that different subunit exchange pathways were dominant for the wild-type and the disease-related variant. Importantly, we also observed that dissociation of wild-type transthyretin was accelerated in the presence of L55P, suggesting the existence of interactions between the two proteins.

MATERIALS AND METHODS

Sample Preparation—Recombinant wild-type and L55P transthyretin were expressed in Escherichia coli as described previously (24). Isotopically labeled transthyretin was expressed in minimal medium with [15N]ammonium chloride and [13C]glucose (Spectra Stable Isotopes Inc.) as the sole nitrogen and carbon sources respectively. All four proteins were purified as previously described (24), with the exception that dialysis was used in place of ion exchange chromatography. Four different solutions were analyzed: wild-type [12C-14N]transthyretin, wild-type [13C-15N]transthyretin, L55P [12C-14N]transthyretin + L55P [13C-15N]- transthyretin, wild-type [12C-14N]transthyretin + L55P [13C-15N]- transthyretin, and L55P [12C-14N]transthyretin + wild-type [13C-15N]- transthyretin. Tris(2-carboxyethyl)phosphine was used as the reducing agent at a concentration of 7 mM where necessary. All chemicals were purchased from Sigma (Poole, Dorset, UK) unless otherwise stated.

Mass Spectrometry—Immediately prior to analysis, proteins were buffer-exchanged into 200 mM ammonium acetate, pH 7.0 using Micro Bio-spin columns (Bio-Rad). A concentration of 7 μM for each protein tetramer was used for all experiments as determined by UV absorbance spectroscopy (extinction coefficient $E_{280} = 7.76 \times 10^4$ M$^{-1}$ cm$^{-1}$) (25) and verified by amino acid analysis (Protein and Nucleic Acid Chemistry Facility, Department of Biochemistry, University of Cambridge). Nano-flow electrospray mass spectra were recorded on an LCT (orthogonal time of flight) mass spectrometer with a Z-spray source (Waters, Manchester, UK). Typically, 2 μl of solution containing transthyretin was electrospayed from gold-coated glass capillaries prepared in-house as previously described (26). To preserve the non-covalent interactions in the transthyretin tetramer, the MS parameters used were: capillary voltage, 1.7 kV; sample cone, 80 V; cone gas, 130 liters h$^{-1}$; ion transfer stage pressure, 5.50 millibars; time-of-flight analyzer pressure 2.15 × 10$^{-6}$ millibars. Under these conditions, no dissociation of the tetramer was observed in the mass spectrum. All spectra were calibrated externally using a solution of cesium iodide (100 mg ml$^{-1}$) and processed with Masslynx 3.4 software (Waters, UK). Spectra are shown here with minimal smoothing and without background subtraction. The relative abundance of the different types of tetramer was calculated from the intensities of both the 14+ and the 15+ charge states and expressed as a percentage of the total intensity of the peaks assigned to the tetramers. All graphs were produced using SIGMAPLOT 2001, version 9.0 (Systat Software UK Ltd., London, UK). The modeling software was produced in-house and was written in the C++ programming language.

RESULTS

Mass Spectrometry of an Isotopically Labeled Transthyretin System—We examined an equimolar solution of wild-type transthyretin containing protein with natural abundance isotopes and protein uniformly labeled with $^{13}$C-$^{15}$N under MS conditions designed to maintain non-covalent interactions (Fig. 2a). The difference in mass between the labeled and unlabeled protein was ∼3 kDa, enabling us to resolve these species in spectra, with peaks corresponding to the 14+ to 16+ charge states of $^{12}$C-$^{14}$N and $^{13}$C-$^{15}$N intact homotetramers. In addition, peaks at high $m/z$ values were assigned to a range of higher oligomers. The masses calculated for these species were consistent with the association of two, three, and four tetramers, each containing a distribution of labeled and unlabeled tetramers.

After 27 h, new peaks were observed between the homotetramer peaks corresponding to tetramers with 1:3, 2:2, and 3:1 (labeled:unlabeled) subunits (Fig. 2b). These heterotetramers occurred as a result of subunit exchange, which resulted in five possible tetramers with unique masses. By measuring the total intensity of peaks within both the 14+ and 15+ charge states of $^{13}$C-$^{14}$N and $^{13}$C-$^{15}$N intact homotetramers. In addition, peaks at high $m/z$ values were assigned to a range of higher oligomers. The masses calculated for these species were consistent with the association of two, three, and four tetramers, each containing a distribution of labeled and unlabeled tetramers.
proteins also allowed us to investigate the effect of the isotopic labeling on the subunit exchange process.

By comparing the rate of decay of homotetramers in an equimolar solution of labeled and unlabeled wild-type or L55P transthyretin, we found that the rates were indistinguishable within the experimental error (data not shown). The results of these investigations into the decay of the homotetramers allowed us to establish optimum conditions for our subsequent reactions at 4 °C and to conclude that isotopic labeling of either protein had no impact on the rate of subunit exchange.

Comparison of L55P Subunit Exchange with That of the Wild Type—
We monitored subunit exchange in an equimolar solution of wild-type transthyretin containing protein with natural abundance isotopes and protein uniformly labeled with $^{13}$C and $^{15}$N. 9 h after initiating the subunit exchange reaction, additional peaks in the mass spectrum corresponding to 1:3, 2:2, and 3:1 ($^{13}$C-$^{15}$N:$^{12}$C-$^{14}$N) heterotetramers were observed (Fig. 4a). As the homotetramer peaks decreased in intensity, with time these additional peaks increased, with the 2:2 peaks remaining at a higher intensity than the 1:3 and 3:1 throughout. After 68 h, no further change was seen, and the intensity of the peaks (0:4, 1:3, 2:2, 3:1, and 4:0) was found to approximate the expected binomial distribution (1:4:6:4:1). We therefore assumed that at this time the reaction was approaching equilibrium.

To investigate the effect of the L55P mutation on subunit exchange, we carried out analogous experiments to those described above using equimolar solutions of $[^{12}$C-$^{14}$N]L55P and $[^{13}$C-$^{15}$N]L55P-transthyretin (Fig. 4c). After just 18 min of incubation, peaks corresponding in mass to 2:2 heterotetramers could be observed, which remained notably more intense than peaks corresponding to the 1:3 or 3:1 species in the early stages of the reaction. After the first hour, the peaks assigned to the 2:2 tetramer were more intense than those of the homotetramers. After 5 h, the reaction approached equilibrium, and the spectrum acquired after 24 h showed the statistical distribution of the 5 tetramers.

Comparison of the results for the variant and wild-type protein revealed that subunit exchange was more rapid for the L55P transthyretin. Minimal subunit exchange occurred before 9 h in the case of the wild-type protein, whereas the 2:2 tetramer was observed after just 18 min for the L55P protein. Importantly, we found that, for the L55P variant, the 2:2 tetramer formed significantly faster than the 1:3 or 3:1 species. By contrast, for the wild-type protein, the 2:2 tetramer was formed at a similar rate to the 1:3 or 3:1 tetramers. In summary, we
found that the L55P tetrameric protein not only exchanged significantly faster than the wild-type but also the rate of production of the 2:2 tetramer was increased, indicating that L55P subunit exchange proceeded via a different pathway to that of the wild-type.

Kinetic Modeling Revealed Mechanisms of Subunit Exchange—To investigate possible mechanisms of subunit exchange, we plotted the intensity of the various homo- and heterotetramers recorded for the two experiments as a function of time. From the mass spectra, it was apparent that the peaks assigned to the tetramers with 2:2 and 3:1 subunits were clearly resolved from neighboring peaks, whereas those for 1:3 tetramers were not. We therefore focused our investigation on the changes in populations of tetramers containing 0:4, 2:2, and 3:1 labeled to unlabeled subunits. The clear difference in the relative rates of formation of the 2:2 and 3:1 tetramers observed in both wild-type and L55P exchange reactions meant that a simple model, involving dissociation of the tetramer to its component monomers followed by rapid reassociation as employed previously (23), would not be able to explain these differences. We considered instead a scenario in which exchange occurred via the dissociation of a single monomer from the tetramer (leaving a trimer) or dissociation of the tetramer to form two dimers via the disruption of either the hydrophobic or β-sheet interfaces (Fig. 1, A and B, respectively). In all cases the dissociation of the tetramer was taken to be the rate-limiting step as suggested by earlier studies (20, 21, 23), with subsequent rapid reassociation of either monomers with trimers or dimers with dimers. Further details of the model, including equations, can be found in the supplemental material.

In the first instance, we considered only the dissociation to monomer and trimer, which proceeded with a rate constant of $k_{d,mon}$, setting the two dimer dissociation rate constants $k_{d,dimA}$ and $k_{d,dimB}$ to zero. This model, however, was unable to account for the experimental finding that the rate of formation of the 2:2 tetramer was more rapid than that of the 3:1 tetramer (supplemental Fig. 1, S1 and S2). The next model we
considered allowed only exchange via both dimer interfaces, setting \( k_{d,\text{dim}} \) to zero (allowing exchange across only one dimer interface would not lead to the formation of the 1:3 and 3:1 species). Comparison of this model with the experimental data indicated that, although a reasonable fit can be achieved for the L55P, the model overestimates the rate of formation of the 2:2 with respect to the 3:1 for the wild-type protein (supplemental Fig. 1, S3 and S4). We therefore considered exchange of both dimers and monomers between tetramers, allowing all three of the rate constants to vary independently. This model was in close agreement with the experimental data for both the wild-type and L55P proteins (Fig. 4, e and f). Interestingly this model achieves the best agreement with the experimental data when dissociation of the tetramer across only one of the dimer interfaces is significant, i.e. when either \( k_{d,\text{dimA}} \) or \( k_{d,\text{dimB}} \) is set to zero.

From these fits, we extracted two rate constants, one for dissociation of tetramers via monomers \( (k_{d,\text{mon}}) \) and one for dissociation of tetramers to dimers formed by the disruption of only one of the two interfaces \( (k_{d,\text{dim}}) \). For wild-type transthyretin, the rate constants \( k_{d,\text{mon}} \) and \( k_{d,\text{dim}} \) were 0.0006 ± 0.00003 min\(^{-1}\) and 0.0005 ± 0.00003 min\(^{-1}\), respectively. For the L55P variant, \( k_{d,\text{mon}} \) and \( k_{d,\text{dim}} \) were determined as 0.0075 ± 0.0003 min\(^{-1}\) and 0.011 ± 0.0003 min\(^{-1}\), respectively. Comparison of these rate constants demonstrated that the dissociation of a tetramer to a monomer was 12.5-fold faster for the L55P tetramer than for the wild-type protein, whereas dissociation to two dimers was 22-fold faster for the variant. This indicates that, for the wild-type tetramer, exchange via monomers and dimers occurs at similar rates, with a marginal preference for the exchange of monomers. For L55P, both reactions were accelerated compared with the wild type, and exchange of dimers between tetramers was faster than that of monomers.

To test the validity of our model, simulated mass spectra of the wild-type and L55P tetramers deduced from the proportions of labeled and unlabeled tetramers were compared at representative time points (Fig. 4, b and d). The results showed that the relative intensities of the peaks in the simulated spectra within each charge state were closely similar to the experimental data. Specifically, the model predicts the ratio of the 1:3, 2:2, and 3:1 tetramers for the wild-type protein after ~24 h and the prevalence of the 2:2 dimers over other heterotetramers after ~1 h of
exchange of the L55P variant. The fact that the model is capable of predicting the tetramer populations implies that the kinetic parameters we have deduced are an accurate description of the subunit exchange that is occurring.

**Formation of Hybrid Tetramers Was Faster than Anticipated**—To investigate the mechanism of subunit exchange of hybrid tetramers, we monitored an equimolar solution of L55P and isotopically labeled wild-type transthyretin (Fig. 5). Interestingly, although the spectra from the homogeneous systems investigated in the previous section were largely symmetrical (with the intensity of the peaks assigned to 3:1 being approximately equal to those 1:3 tetramers and similarly for the 4:0 and 0:4 species), this symmetry was absent from the spectra of the heterogeneous system. First, the peaks corresponding to the L55P homotetramer (0:4) were considerably less intense than those assigned to the wild-type homotetramer (4:0) throughout the time course, despite the fact that they had very similar intensities initially. Second, for early time points, the 1:3 wild-type L55P was more intense than the 3:1, but at later time points, this situation was reversed. After 22 h, the intensities of the tetramers did not change significantly, and we assumed that the subunit exchange reaction was approaching equilibrium.

To extend our model to explore the formation of hybrid tetramers, we employed the dissociation rate constants \( k_{d,\text{dim}} \) and \( k_{d,\text{mon}} \) determined from the wild-type/wild-type and L55P/L55P exchange reactions. These values were then used to produce \( k_{d,\text{dim}} \) and \( k_{d,\text{mon}} \) for each of the 14 possible hybrid tetramers, with the assumption that the rate of dissociation correlates directly with the number of L55P subunits (27) (supplemental Table). Comparison of the predicted data obtained using the rate constants derived from the homotetramer exchange reactions provided a surprisingly good fit to the subunit exchange kinetics measured for wild-type and L55P proteins in the same solution (Fig. 5a).

Specifically, the rate of decay of the L55P homotetramer was closely similar to the rate measured previously, and the data for formation of the 1:3 and 3:1 tetramers were in reasonable agreement with the model. Surprisingly, however, the rate of formation of the 2:2 hybrid tetramer was faster during the early time points of the reaction than predicted by our model. Moreover, from our data, the 2:2 tetramer formed faster than the 1:3 tetramer, whereas the model predicted formation of the 1:3 tetramer and 2:2 tetramer at closely similar rates. When we used this model to predict populations of different tetramers and compared this with mass spectra recorded at selected time points (Fig. 5a) these differences were again apparent. In summary therefore, we found that at all of the time points in the reaction rates derived from the homogeneous transthyretin reactions were not consistent with the increased rate of formation of the 2:2 hybrid tetramer when L55P and wild-type proteins were incubated in the same solution.

We investigated this discrepancy by varying our kinetic parameters. Interestingly, we found that the model was insensitive to changes in the rates of dissociation of the hybrid tetramers and fit the data less well when the rate of dissociation to monomers was increased (data not shown). The critical factor in improving the fit of the model was found to be the rate constant for dissociation of the wild-type homotetramer to dimers. An increase of this rate from 0.0005 to 0.001 min\(^{-1}\) resulted in a close fit to the experimental data (Fig. 5, c and e). Specifically, the revised model successfully predicted both a faster rate of decay for the wild-type homotetramers and more rapid formation of the 2:2 rather than the 1:3 tetramer. This observation suggests that, in solutions containing both proteins, dissociation of wild-type homotetramers to dimers is accelerated by interactions with the L55P variant.

**DISCUSSION**

Using the MS approach described here, we have demonstrated subunit exchange between tetrameric proteins with natural abundance isotopes and those uniformly labeled with \(^{13}\)C and \(^{15}\)N. We have also monitored the formation of hybrid tetramers containing both wild-type and L55P subunits. The wealth of data accrued from these reactions has enabled us to delineate different mechanisms of subunit exchange. Transthyretin is established as one of an increasing number of disparate proteins that undergo subunit exchange reactions (28–32), and given the challenges involved in studying this process, it is likely that the approach described here will be widely applicable to a variety of biological systems.

The experiments described here have allowed us to deduce that the most appropriate model for the subunit exchange process is one in which both dimers and monomers are exchanged between tetramers. We have extracted rate constants for the individual mechanisms to demonstrate that exchange of monomers and dimers occurs at similar rates for the wild-type protein. Importantly however, exchange via dimers was found to be the dominant mechanism for the L55P variant. The two dissociation events can be combined to provide overall rate constants of 0.017 min\(^{-1}\) and 0.0011 min\(^{-1}\) for the L55P variant and wild-type protein, respectively, with the latter being in close agreement with that determined previously for the wild-type protein (0.0015 min\(^{-1}\)) (21).

Interestingly, we have found that the data are consistent with a model that allows exchange of dimers by dissociation across only one of the two possible dimer interfaces. It therefore seems reasonable, in the light of the structural information known, that dimer dissociation occurs via
disruption of the predominantly hydrophobic interface. This is supported by a recent publication that describes the effect of linking wild-type transthyretin subunits across the hydrophobic interface and demonstrates that this prevented subunit exchange (33). We anticipate that the hydrophobic interface would be particularly destabilized under the conditions of our experiment (4 °C), where hydrophobic interactions, in general (34) and in the specific case of TTR (35), are known to be disfavored.

Results indicating that the L55P point mutation causes the rate of subunit exchange to increase by more than an order of magnitude relative to the wild-type protein are intriguing, particularly because previous studies have shown that the V30M mutation has very little effect on the subunit exchange rate of the protein (21). More rapid dissociation and reassociation of monomers from L55P tetramers than from the wild-type protein might be anticipated, given the established propensity of this variant to form amyloid via dissociation to monomers (24). We were surprised, however, to find that exchange of dimers is the dominant mechanism of subunit exchange for the L55P variant. One possible explanation for this observation might be that disulfide-bonded dimers formation between L55P subunits. This possibility was discounted both by the fact that the addition of reducing agent did not affect the rate of exchange (supplemental Fig. 2) and that dissociation of monomers from the tetramer can be induced, under appropriate conditions that disrupt non-covalent interactions, during MS (data not shown). Consequently, rather than the presence of disulfide bonds, these results imply that the L55P point mutation compromises part of the hydrophobic interface, perturbing interactions important for stability and promoting dissociation to dimers in preference to dissociation to monomers.

One of the surprising findings from our study of the L55P wild-type system is the different rates of formation of the two hybrid tetramers linked by symmetry, (i.e. 1:3 and 3:1). Because we have established that the dissociation of hybrid tetramers containing L55P is occurring at a faster rate than that of the wild-type homotetramers, any wild-type subunits that are released by dissociation are rapidly redistributed across a large number of predominantly L55P tetramers (Fig. 6). This essentially makes dissociation of the wild-type protein the rate-limiting step. It is also interesting to note that the 3:1 tetramer, containing three wild-type subunits, is prevalent as the reaction approaches equilibrium. It is established that the thermodynamic stability is greater for wild-type than the L55P variant and that stability correlates directly with the number of subunits of the variant protein (36, 37). To summarize, we have clear evidence that, during the early stages of the reaction the tetramer with one wild-type subunit and three L55P subunits (1wild-type:3L55P) is predominant, when kinetics dictate the reaction products, whereas at equilibrium, when thermodynamic stability is the crucial factor, the 3wild-type:1L55P tetramer population is prevalent.

For the hybrid tetramers, the rate of formation of the 2wild-type:2L55P tetramer is faster than predicted from rate constants derived from the homogeneous solutions. To correct this discrepancy, an explanation for this observation might be that disulfide-bonded dimers is required. This strongly implies interaction between the wild-type homotetramers and the L55P components of the system. A plausible mechanism for such an interaction would be the formation, in solution, of transient oligomers larger than the tetramer. This is in accord with the observation of the higher oligomers in the mass spectrum (Fig. 2). It is also possible that the addition of wild-type protein to a solution containing the aggregation-prone L55P might lead to incorporation of wild-type tetramers into soluble oligomers of L55P. Although the nature of these large transient oligomers is not known, we anticipate that interactions within them may destabilize the hydrophobic interface, leading to an enhanced rate of dissociation of dimers from wild-type transthyretin tetramers. Therefore, when the variant and wild-type proteins are incubated together, our model is consistent with both proteins exchanging via a similar mechanism, presumably as a consequence of interactions within large oligomers.

Given that both wild-type and variant transthyretin are co-expressed in heterozygous individuals (17–19), the interactions of tetramers during their lifetime in plasma (half-life 8–18 h (38)) are important determinants in the fibril formation process. Although it might be anticipated that incorporation of wild-type subunits would have a stabilizing effect on L55P, as observed for T119M in hybrid tetramers with V30M (39), heterozygotes with the L55P mutation develop familial amyloidotic polyneuropathy with the earliest age of onset of all transthyretin-associated diseases, indicating that any protective effect is minimal. Moreover, our results suggest that wild-type homotetramers may be incorporated into transient higher oligomers in the presence of L55P, increasing their propensity to dissociate to dimeric species, which may ultimately become trapped in insoluble aggregates. The fact that these hybrid tetramers and transient oligomers are formed within a physiologically relevant time scale has clear implications for disease mechanisms associated with this mutation and, more importantly, for therapeutic strategies designed to prevent them.

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REFERENCES
1. Selkoe, D. J. (2003) Nature 426, 900–904
2. Saraiva, M. J. (1995) Hum. Mutat. 5, 191–196
3. Plante-Bordeneuve, V., Lalu, T., Misrahi, M., Reilly, M. M., Adams, D., Lacroix, C., and Said, G. (1998) Neurology 51, 708–714
4. Sekijima, Y., Wiseman, R. L., Matteson, J., Hammarstrom, P., Miller, S. R., Sawkar, A. R., Balch, W. E., and Kelly, J. W. (2005) Cell 121, 73–85
5. Sebastian, M. P., Saraiva, M. J., and Damas, A. M. (1998) J. Biol. Chem. 273, 24715–24722
6. Hornberg, A., Olofsson, A., Enqvist, T., Lundgren, E., and Sauer-Eriksson, A. E. (2004) Biochim. Biophys. Acta 1700, 93–104
7. Colon, W., and Kelly, J. W. (1992) Biochemistry 31, 8654–8660
8. Gustavsson, A., Engstrom, U., and Westermark, P. (1991) Biochim. Biophys. Res. Commun. 175, 1159–1164
9. Jiang, X., Smith, C. S., Petraroli, H. M., Hammarstrom, P., White, J. T., Sacchettini, J. C., and Kelly, J. W. (2001) Biochemistry 41, 11442–11452
10. McCutchen, S. L., Colon, W., and Kelly, J. W. (1993) Biochemistry 32, 12119–12127
11. Olofsson, A., Ippel, H. J., Baranow, V., Horstedt, P., Wijmenga, S., and Lundgren, E. (2001) J. Biol. Chem. 276, 39592–39599
12. Matsubara, K., Mizuguchi, M., Igarashi, K., Shinohara, Y., Takeuchi, M., Matsuura, A., Saijo, T., Mori, Y., Shinoda, H., and Kawano, K. (2005) Biochemistry 44, 3280–3288
13. Serag, A. A., Altenbach, C., Gingery, M., Hubbell, W. L., and Yeates, T. O. (2002) Nat. Struct. Biol. 9, 734–739
14. Hornberg, A., Enqvist, T., Olofsson, A., Lundgren, E., and Sauer-Eriksson, A. E. (2000) J. Mol. Biol. 302, 649–669
15. Blake, C., and Serpell, L. (1996) Structure (Lond.) 4, 989–998
16. Serag, A. A., Altenbach, C., Gingery, M., Hubbell, W. L., and Yeates, T. O. (2001) Biochemistry 40, 9089–9096
17. Saraiva, M. J., Sherman, W., Marboe, C., Figueire, A., Costa, P., de Freitas, A. F., and Gawinowicz, M. A. (1990) Scand. J. Immunol. 32, 341–346
18. Murakami, T., Yi, S., Yamamoto, K., Maruyama, S., and Araki, S. (1992) Ann. Neurol. 31, 340–342
19. Thylén, C., Wahlqvist, J., Haertner, E., Sandgren, O., Holmgren, G., and Lundgren, E. (1993) EMBO J. 12, 743–748
20. Bernstein, R., Robbins, J., and Rall, J. (1970) Endocrinology 86, 383–390
21. Schneider, F., Hammarstrom, P., and Kelly, J. W. (2001) Protein Sci. 10, 1606–1613
22. Sekijima, Y., Hammarstrom, P., Matsumura, M., Shimizu, Y., Iwata, M., Tokuda, T., Ikeda, S., and Kelly, J. W. (2003) Lab. Invest. 83, 409–417
23. Wiseman, R. L., Green, N. S., and Kelly, J. W. (2005) Biochemistry 44, 9265–9274
24. Lashuel, H. A., Wurth, C., Wool, L., and Kelly, J. W. (1999) Biochemistry 38, 13560–13573

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25. Miroy, G. J., Lai, Z., Lashuel, H. A., Peterson, S. A., Strang, C., and Kelly, J. W. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15051–15056
26. Nettleton, E. J., Sunde, M., Lai, V., Kelly, J. W., Dobson, C. M., and Robinson, C. V. (1998) J. Mol. Biol. 281, 553–564
27. Hammarstrom, P., Wiseman, R. L., Powers, E. T., and Kelly, J. W. (2003) Science 299, 713–716
28. Bova, M. P., Ding, L. L., Horwitz, J., and Fung, B. K. (1997) J. Biol. Chem. 272, 29511–29517
29. Nelson, S. W., Honzatko, R. B., and Fromm, H. J. (2001) FEBS Lett. 492, 254–258
30. Saxl, R. L., Changchien, L. M., Hardy, L. W., and Maley, F. (2001) Biochemistry 40, 5275–5282
31. Ferreira, S. T., and De Felice, F. G. (2001) FEBS Lett. 496, 129–134
32. Gu, L. X., Abulimiti, A., Li, W., and Chang, Z. Y. (2002) J. Mol. Biol. 319, 517–526
33. Foss, T. R., Kellier, M. S., Wiseman, R. L., Wilson, I. A., and Kelly, J. W. (2005) J. Mol. Biol. 347, 841–854
34. Fersht, A. (2000) in Structure and Mechanism in Protein Science (Julet, M. R., ed) pp. 508–536, W. H. Freeman and Company, New York
35. Hammarstrom, P., Jiang, X., Deechongkit, S., and Kelly, J. W. (2001) Biochemistry 40, 11453–11459
36. Hammarstrom, P., Sekijima, Y., White, J. T., Wiseman, R. L., Lim, A., Costello, C. E., Alland, K., Garzuly, F., Budka, H., and Kelly, J. W. (2003) Biochemistry 42, 6656–6663
37. Lashuel, H. A., Lai, Z. H., and Kelly, J. W. (1998) Biochemistry 37, 17851–17864
38. Benson, M. D., Klave-Beckerman, B., Liepnieks, J. J., Murrell, J. R., Hanes, D., and Uemichi, T. (1996) The Nature and Origin of Amyloid Fibrils (Bock, G., Goode, J., and Costa, P., eds) John Wiley and Sons, Inc., New York
39. Hammarstrom, P., Schneider, F., and Kelly, J. W. (2001) Science 293, 2459–2462
40. Wojtczak, A., Neumann, P., and Cody, V. (2001) Acta Crystallogr. 57, 957–967