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New Concepts

Protein Conformation and Disease: Pathological Consequences of Analogous Mutations in Homologous Proteins†

Fred J. Stevens,* Phani R. Pokkuluri, and M. Schiffer

Biosciences Division, Argonne National Laboratory, Argonne, Illinois 60439

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ABSTRACT: The antibody light chain variable domain (V_L)[1] and myelin protein zero (MPZ) are representatives of the functionally diverse immunoglobulin superfamily. The V_L is a subunit of the antigen-binding component of antibodies, while MPZ is the major membrane-linked constituent of the myelin sheaths that coat peripheral nerves. Despite limited amino acid sequence homology, the conformations of the core structures of the two proteins are largely superimposable. Amino acid variations in V_L account for various conformational disease outcomes, including amyloidosis. However, the specific amino acid changes in V_L that are responsible for disease have been obscured by multiple concurrent primary structure alterations. Recently, certain demyelination disorders have been linked to point mutations and single amino acid polymorphisms in MPZ. We demonstrate here that some pathogenic variations in MPZ correspond to changes suspected of determining amyloidosis in V_L. This unanticipated observation suggests that studies of the biophysical origin of conformational disease in one member of a superfamily of homologous proteins may have implications throughout the superfamily. In some cases, findings may account for overt disease; in other cases, due to the natural repertoire of inherited polymorphisms, variations in a representative protein may predict subclinical impairment of homologous proteins.

The rapidly accelerating accumulation of genomic data combined with growing knowledge of protein three-dimensional structures has motivated much recent work in analysis of protein homology. Such studies range from determination of evolutionary relationships to identification of templates for prediction of protein structure. Well-known examples of the resulting databases include CATH (*1*), SCOP (*2*), HSSP (*3*), and COG (*4*). We anticipate that awareness of protein homology, prompted by these and other emerging tools, will accelerate cross-functional integration of relationships between protein structure and physicochemical properties. Such integration should significantly contribute to our understanding of the structural basis of molecular disease.

The category of pathology termed "conformational disease" (*5*−*16*) was recently introduced to designate maladies that result from conformational transitions in proteins that lead to self-assembly or to aggregation. Such diseases include the prion-based spongiform encephalopathies, various amyloidoses, Huntington's disease, Parkinson's disease, pancreatic cell death in adult-onset diabetes, cirrhosis, and emphysema resulting from serpin aggregation, and, possibly, Alzheimer's disease. With the possible exception of some prion diseases such as mad cow disease and kuru, conformational diseases are not thought to be transmissible. In some cases, the

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* To whom correspondence should be address. Phone: 630-252-3837. Fax: 630-252-5517. E-mail address: fstevens@anl.gov.

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responsible protein or protein fragment has the normal primary structure. In other instances, the risk for a particular conformational disorder is enhanced by somatic mutations or by the inheritance of genetic polymorphisms that apparently destabilize the protein and enhance its likelihood of unfolding and aggregating. However, conformational diseases are distinguished from other genetic pathologies that originate from polymorphisms that inactivate a protein or cause it to be unable to fold.

In this report, we describe an example in which sites of mutation that account for conformational diseases in one protein are observed as sites of variations that cause genetic disease in a homologous protein of unrelated function. This observation suggests that studies of conformational or genetic disease may benefit from a broader perspective that includes consideration of structural variations of physiological and physicochemical significance throughout a family of homologous proteins.

Immunoglobulin Superfamily. The immunoglobulin superfamily is a diverse cohort of proteins that are homologous to the motifs of antibody variable and constant β domains. The critical physiological contributions of the superfamily extend beyond immunity to include the roles of adhesion molecules, growth factor receptors, and immunoglobulin receptors (*17*). Myelin protein zero (MPZ;[1] P0) is the most abundant protein of the myelin sheath. It has approximately 25% amino acid sequence homology to the antibody variable domain (Figure 1) with most of the homology occurring in regions of the molecule that determine the internal packing of the domain. Positions at which amino acid segments have been inserted or deleted in the MPZ sequence relative to the reference κ4 V_L sequence correspond to sites of length variation among human V_Ls.

Superfamily Diseases of Structural Origin. Dejerine-Sottas syndrome (DSS) is a severe demyelinating peripheral neuropathy (*18*−*20*); Charcot-Marie-Tooth disease (CMT), also known as chronic demyelinating neuropathy (*21*−*25*) is less severe. CMT results from gene duplication in some cases and point mutations of MPZ and other proteins. While both diseases can occur as a result of point mutations; the mutations associated with DSS appear to have the potential to evoke the more drastic physicochemical consequences. The diagnosis of DSS occurs during infancy, whereas CMT is usually detected in the second or third decade of life. Approximately, 40 mutations in MPZ have been documented to date (Table 1). To our knowledge, the molecular consequences of these mutations have not been determined.

Cumulative point mutations within the V_L appear to be the dominant determinant of the pathogenic potential of light chains produced during multiple myeloma and primary amyloidosis (*26*−*29*, *41*, *42*). The conformational diseases of antibody light chains include amyloid (AL) fibril deposition, nonfibrillar light chain deposition disease (LCDD), and amorphous tubular cast nephropathy. If the light chain crystals found in proximal tubular cells are the agents of tubular dysfunction in some cases of light chain-related acquired Fanconi's syndrome, then this pathology may also be considered a protein conformational disorder.

Structural Analogy in V_L and MPZ Diseases. Figure 1b compares the structure of a human κ4 antibody light chain variable domain (*30*) with MPZ. Despite only approximately 25% sequence homology, the structural correspondence of the core unit is clear; major divergences are restricted to the loops corresponding to the complementarity-determining regions in the antibody V_L. A few important structural determinants found in most members of the immunoglobulin superfamily are highlighted. These include the cysteine residues that form a disulfide bond between positions 23 and 88, isoleucine at position 75, and arginine and aspartic acids at positions 61 and 82, which form a buried salt bridge. The corresponding residues in MPZ are Cys21, Cys98, Ile85, Arg69, and Asp92.

A single-site mutation of Cys98 in MPZ is responsible for an inherited form of DSS (*19*). Several alterations of Arg69 have been documented in CMT (*23*, *31*−*36*). The replacement of Ile85 with Thr occurred in a patient whose MPZ also exhibited two additional variations, Asn87His and Asp99Asn (*18*).

No mutation of either cysteine residue involved in the intradomain V_L disulfide bond has been observed in a naturally occurring soluble-free antibody light chain. However, light chains in which Cys is mutated have been documented. Our database of some 370 sequences of V_L produced during plasma cell diseases includes one missense mutation of Cys88 in a κ cDNA and three examples of missense mutations of Cys23 in λ cDNA, one of which was obtained from a patient with amyloidosis (*37*). Cys mutations are found in functional antibodies, in which the heavy chain effectively serves as a chaperone; for instance, a human antibody that binds HIV p25 in which Tyr replaced Cys23 has been described (*38*). The origin of the inability of a free light chain with a mutated disulfide bond to survive as a soluble protein has been experimentally determined. Replacing Cys23 with Val caused a 4.2 kcal/mol destabilization (*39*) (corresponding to a 1000-fold increase in relative concentration of an unfolded form). To accomplish this experiment required first introducing an enabling mutation (Tyr32His) that increased the stability of the variable domain by 1.2 kcal/mol. The destabilization resulting from the replacement of Cys with Tyr rather than Val is likely to exceed 4.2 kcal/mol due to the steric problems created by the larger bulk of the Tyr side chain as well as by the introduction of a polar group into the hydrophobic core. These results from V_L-based studies support the probability that the Cys98Tyr replacement in MPZ sufficiently destabilizes the protein to render it ineffectual in contributing to the myelin sheath, causing DSS.

In contrast to the rareness of Cys mutations, change of Arg61 is quite common in κ V_L. Approximately 10% of the κ sequences in our database exhibit replacement of Arg with a polar residue. Of these 15 proteins, 12 were amyloidogenic, one of which also formed crystals in the kidney tubules (A. Solomon, University of Tennessee Medical Center/Knoxville, unpublished results), and no clinical information was provided for three. Interestingly, only one mutation of Arg61 has been observed in free λ, a replacement with Gln in an amyloid-forming protein. This may relate to a lower average stability of λ compared to κ light chains, which would be consistent with the higher rate of amyloid formation by λ V_L and suggest that the consequences of the substantially

[1] Abbreviations: AL, amyloid; CMT, Charcot-Marie-Tooth disease; DSS, Dejerine-Sottas syndrome; LCDD, light chain deposition disease; MPZ, Myelin protein zero; PZR, protein zero related; V_L, light chain variable domain.

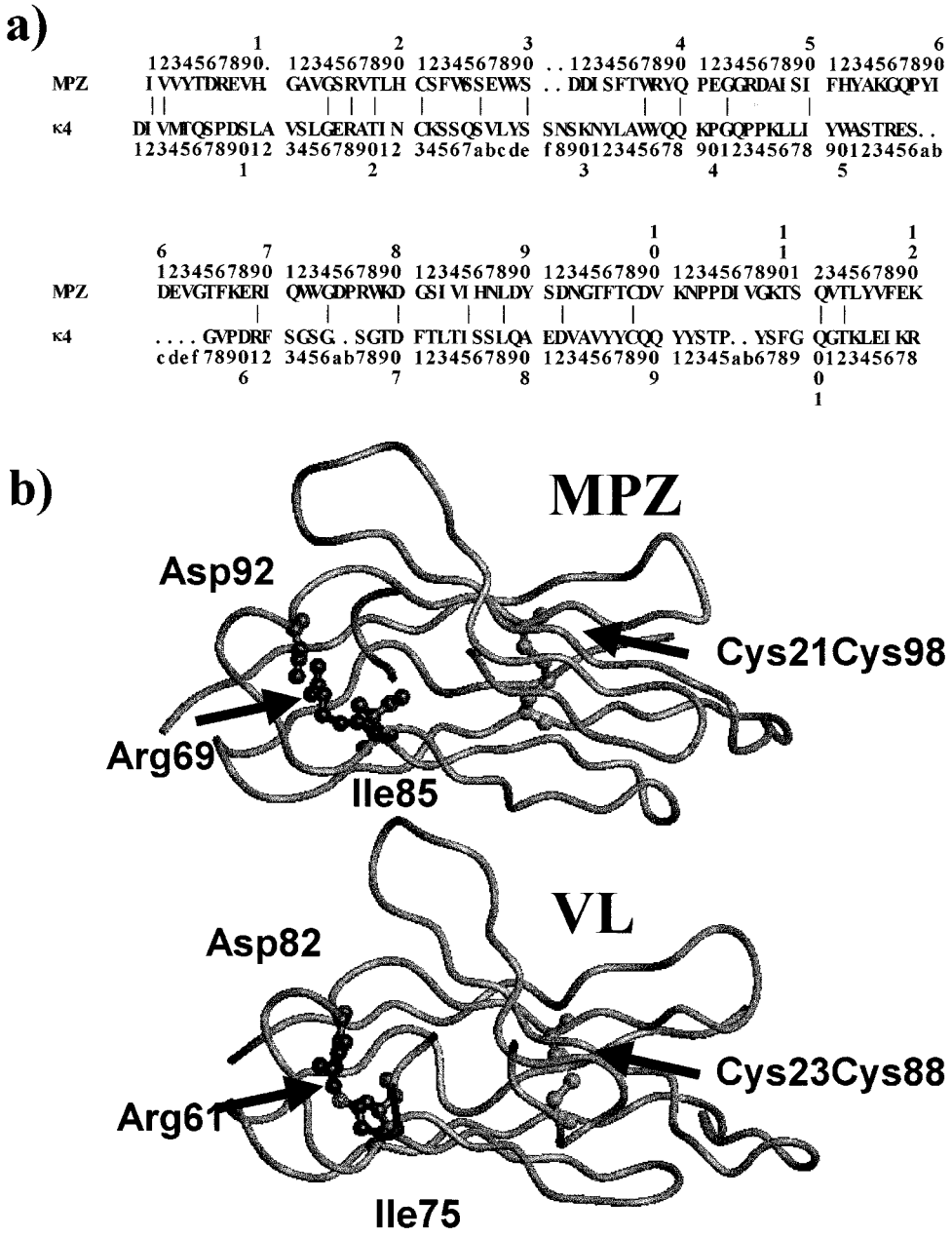

FIGURE 1: (a) Sequence comparison of aligned primary structures of human myelin protein zero and a human κ4 protein. The protein was produced in large quantities by a patient with no pathological consequences (*77*) and its structure has been determined (*30*). (b) V_L (*30*) and MPZ (*78*) structures showing positions of Arg61, Asp82, Cys23/88, Ile75, and MPZ equivalents Arg69, Asp89, Cys21/98, and Ile85. Coordinates are 1LVE and 1NEU, respectively.

destabilizing loss of the Arg61−Asp82 salt bridge are not tolerated. It remains to be determined whether polar replacements of Arg69 in MPZ lead to an impaired protein or a form that is completely dysfunctional. However, the phenotypic association with CMT rather than the more severe DSS suggests that the result is a destabilized but functional protein.

Mutations of Ile75 in λ V_L occur in about 8% of the available sequences; all, however, are reasonably conservative replacements by Leu or Val. Mutations of Ile75 in κ V_L are found in 5% of the primary structures, for which only two have clinical data available. Of the six sequences, five are from cDNA rather than protein, possibly implying severe instability of κ V_L that contain alternative residues at this position. In two proteins, Ile75 has been found to be deleted; both proteins were amyloidogenic. Ile75 and neighboring Leu73 are important contributors to the hydrophobic packing of the domain. Introduction of Thr at position 75 or at position 73, as seen in another CMT-related MPZ polymorphism (*40*), can be expected to significantly destabilize the protein both through its smaller size, creating an internal cavity, and by the introduction of a polar group with unsatisfied hydrogen-bonding potential into a nonpolar environment. On the basis of these results obtained in V_L studies, it seems likely that of the three variations observed in the MPZ characterized by Warner et al. (*18*), the most likely to account for DSS was the Ile85Thr transition.

Although the extensive primary structure variation of V_L has obscured the explicit conformational origin of light chain diseases, a few generalities are emerging. For instance, light chains found in AL are of impaired stability (*27*, *41*, *42*) and can be broadly considered in two categories. The first category is characterized by the presence of one or more

Table 1: Amino Acid Variations Associated with DSS and CMT

variation	path	structure[b]	LC equiv κ-IV	ref
I1M[a]	CMT1B	I1		*50*
T5I	CMT1	T5	Q6	*51*
S15F[a]	CMT2	S15	E17	*52*
C21A	CMT1B	C23	C23	*53*
S25P[a]	CMT1	S25	Q27	*54*
V29F	CMT1	V29		*40*
I33F[a]	CMT1B	I33	N31	*55*
S34C[a]	DSS	S34	Y32	*56, 57*
F	CMT1			*56*
D	CMT1B			*56, 58*
F35Δ[a]	CMT1B	F35	L33	*59*
R45Q	CMT1B	R45		*60*
S49L[a]	CMT1B	S49	L47	*61, 62*
H52R	CMT1B	H52	W50	*63*
Y53C	CMT1	Y53		*64*
	DSS			*61*
D61E[a]	CMT1B	D61		*65*
G	CMT1B			*66*
G64E	CMT1B	G64		*67*
K67E[a]	CMT1B	K67		*65, 68*
R69H	CMT1B	R69	R61	*23, 32*
C	CMT1B			*23, 32*
S	CMT1B			*35*
P	CMT1B			*34*
W72C	CMT1B	W72	G64	*69*
D80N	CMT1B	D80	D70	*47*
I83T	CMT1	I83	L73	*40*
I85T	DSS	I85	I75	*18*
N87H	DSS	N87	S77	*18*
N93S[a]	CMT1B	N93	V83	*62*
A	CMT1B			*53*
T95M[a]	CMT1	T95	V85	*70*
	CMT2			*71*
C98Y	DSS	C98	C88	*19*
D99N	DSS	D99	Q89	*18, 72*
K101R	CMT	K101	Y91	*73*
K102P[a]	CMT1B	N102	Y92	*74*
P103L	CMT1			*40*
I105T	CMT1B			*75*
I106T[a]	CMT1			*76*
L[a]	CMT1			*73*
G108S[a]	CMT1	G108		*76*

[a] Reported position decreased by 29 units for alignment with the MPZ structural model (*78*). [b] Equivalent position in 3D-structure of rat MPZ (*78*).

highly destabilizing variations that are correlated with amyloidosis. These mutations include loss of arginine at position 61, loss of isoleucine at position 75, gain of aspartic acid at position 31, or introduction of a glycosylation acceptor site at any of several locations (*29*). The second category represents a stochastic outcome; the probability of fibrillogenesis is the cumulative result of the intrinsic stability of the germline-encoded V_L plus the contributions of each random variation, some of which are destabilizing, stabilizing, or of little consequence.

V_L studies have found that many amino acid variations have little affect or improve or impair stability by less than approximately 1 kcal/mol. This suggests that there may exist analogous genetic polymorphisms in MPZ that have not been documented to date, chiefly because the consequences are not sufficiently profound to produce an obvious clinical problem. It is reasonable to assume that some variations destabilize moderately. No immediate consequences might be observed from polymorphisms that introduce intermediate stabilization or destabilization.

CONCLUSIONS

Both antibody variable and constant domains are capable of fibril formation. This likely results from vulnerability of the β domain structure to significant stability modifications from limited amino acid substitutions as well as its propensity to achieve an alternative conformation necessary for fibril assembly. Both domains represent archetype structures of the immunoglobulin superfamily, which in addition to the antibodies, T-cell receptors, and other proteins related to maintenance of the immune system, also comprises a large number of cell-adhesion proteins and cell-surface receptors. All of these proteins are subject to polymorphisms; variations in the primary structure of other family members are also linked to disease processes. For instance, impaired surface expression of immunoglobulin on B cells during chronic lymphocytic leukemia is associated with several different defects in B29 (CD79b) genes (*43*). A newly identified protein, protein zero related (PZR), is of unproven function (*44*, *45*). However, it is abundant in a number of tissues, including heart and kidney. It is suggested that PZR is a substrate for tyrosine phosphatase SHP-2 (44), which is a regulator of cell migration and adhesion (*46*). PZR and MPZ share most of the amino acid homologies discussed here, and we can anticipate that homologous polymorphisms could lead to analogous dysfunctions. Interestingly, PZR is reported to have a Gln at the position corresponding to MPZ Asp92, and thus the salt bridge with Arg69 may be replaced by a weaker ionic interaction. No mutation that introduces Gln at the corresponding position 82 has been observed in disease-related light chains; its consequences can be addressed by construction of the mutant in a recombinant V_L (*27*).

Although most instances of CMT appear to involve demyelination, a recently reported case was found in a patient who exhibited thickened myelin sheaths (*47*). The MPZ was found to incorporate a variation that replaced Asp80 with asparagine. In most κ V_L, the corresponding position is Asp70—all human κ V_L germline genes encode an acidic residue at this position. Asn70 has been observed in the primary structure of eight V_Ls from plasma cell disease patients. All examples to date are from κ germline genes; seven proteins formed amyloid, one deposited as LCDD. The side chain of position 70 is exposed to solvent and does not appear to contribute significantly to the stability of the domain. Thus, replacement of Asp70 with asparagine would not be obviously destabilizing. The finding of Lagueny et al. (*47*) that this variation might be responsible for CMT suggests that the homologous mutation in the light chain V_L could contribute to fibril formation through an unusual mechanism. Experiments are now underway to determine whether the Asp70Asn interchange enhances an assembly interaction, rather than significantly decreases the stability of the domain.

A large number of mutations in any of several C-like (I motif) domains in L1 cell adhesion molecule (L1CAM) impair its role in development of the nervous system. L1CAM polymorphisms lead to various forms of mental retardation and physical abnormalities (for review, see ref *48*). Several of the reported L1CAM polymorphisms remove a conserved Cys or introduce an inappropriate Cys, an observation also noted in variants of the fibroblast growth

factor, an immunoglobulin superfamily representative for which genetic errors are associated with faulty skeletal development (*49*).

The finding that studies of antibody light chain amyloidosis are relevant to understanding various inherited neuropathies, and potentially other diseases, was unexpected. Although the immunoglobulin superfamily contains many structurally homologous proteins, each member of the family has its own unique features. Homology analyses allow analogous dysfunction-engendering consequences of polymorphisms at key structural features to be anticipated.

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