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The BRICHOS Domain, Amyloid Fibril Formation, and Their Relationship

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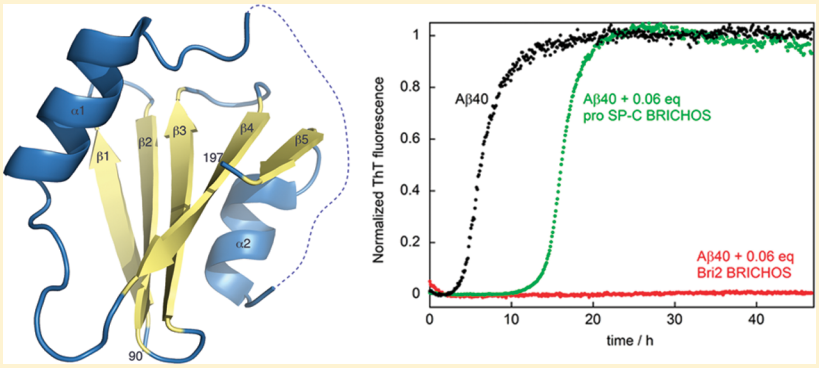

ABSTRACT: Amyloid diseases are defined by tissue deposition of insoluble, fibrillar β-sheet polymers of specific proteins, but it appears that toxic oligomeric species rather than the fibrils are the main cause of tissue degeneration. Many proteins can form amyloid-like fibrils *in vitro*, but only ∼30 proteins have been found to cause mammalian amyloid disease, suggesting that physiological mechanisms that protect against amyloid formation exist. The transmembrane region of lung surfactant protein C precursor (proSP-C) forms amyloid-like fibrils *in vitro*, and SP-C amyloid has been found in lung tissue from patients with interstitial lung disease (ILD). ProSP-C contains a BRICHOS domain, in which many ILD-associated mutations are localized, and the BRICHOS domain can prevent SP-C from forming amyloid-like fibrils. Recent data suggest that recombinant BRICHOS domains from proSP-C and Bri2 (associated with familial dementia and amyloid formation) interact with peptides with a strong propensity to form β-sheet structures, including amyloid β-peptide associated with Alzheimer's disease. Such interactions efficiently delay formation of fibrils and oligomers. The BRICHOS domain is defined at the sequence level and is found in ∼10 distantly related proprotein families. These have widely different or unknown functions, but several of the proteins are associated with human disease. Structural modeling of various BRICHOS domains, based on the X-ray structure of the proSP-C BRICHOS domain, identifies a conserved region that is structurally complementary to the β-sheet- and/or amyloid-prone regions in the BRICHOS domain-containing proproteins. These observations make the BRICHOS domain the first example of a chaperone-like domain with specificity for β-prone regions.

Misfolding of proteins underlies amyloid diseases, in which specific polypeptides form β-sheet polymers in which the same interactions are seen over and over again in a highly repetitive manner.[1] Amyloid is proposed to be a generic protein structure because proteins and peptides of many sequences have been observed to change from their natively unfolded or folded structure into amyloid fibrils depending on the solution conditions.[2] Almost all proteins contain segments that have the potential to form amyloid-like fibrils.[3] So far, ∼30 amyloid diseases are known, among them Alzheimer's disease (AD), prion diseases, and type II diabetes mellitus.[4] More amyloid diseases are likely to be found, although the fact that only ∼0.1% of the proteins in the human proteome has been found to form amyloid *in vivo* implies that nature has found ways to prevent aggregation into amyloid for the main part of the proteome. Proposed protective mechanisms are burial of amyloidogenic segments in the protein interior, the action of general molecular chaperones, and self-regulatory elements in certain proteins.[5]

In AD, fibrils are formed from the amyloid β-peptide (Aβ), and in particular, oligomeric assemblies smaller than fibrils are toxic and can trigger neuronal dysfunction and have even been found to impair the retrieval of learned memories.[6,7] There is today no

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cure for AD, and symptomatic therapies are being used with limited effects. Treatment strategies have so far focused on decreasing the amount of aggregating protein [mainly by inhibiting secretase enzymes that generate Aβ from the amyloid β precursor protein (APP)], inhibition of Aβ misfolding, and elimination of the toxic forms of peptides by immunotherapies, but the results of clinical trials have been disappointing.[8] Currently, the only amyloid disease for which there exists a disease-modifying therapy is polyneuropathy due to transthyretin deposition, in which case substances that stabilize the natively folded transthyretin slow the progress of the disease.[9]

THE BRICHOS DOMAIN AND ITS PARENT PROPROTEINS

The BRICHOS domain, first described in 2002, contains ~100 amino acid residues, has a unique fold, and is present in a diverse set of proteins found in species from humans to marine organisms like echinoderms and amphioxus.[10−12] The name BRICHOS is derived from the three proteins, Bri2 [a member of the Bri family also termed integral transmembrane protein (ITM)], chondromodulin-I [ChM-I, also known as leukocyte cell-derived chemotaxin (LECT)], and SP-C (Figure 1). Other

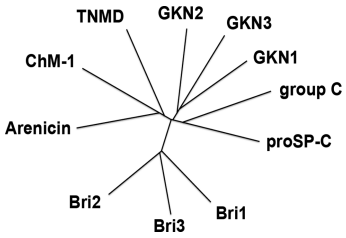

Figure 1. Evolutionary tree of the BRICHOS domain family. The different BRICHOS domain-containing proteins are positioned such that the distances between them reflect their evolutionary separation. Arenicins are so far found only in worms, and GKN3 is not expressed as a functional protein in humans;[62] however, all other BRICHOS domain-containing proteins are found in humans. Abbreviations: ChM, chondromodulin; GKN, gastrokine; TNMD, tenomodulin.

BRICHOS domain-containing proteins encompass gastrokines (GKN),[13] tenomodulins (TNMDs, also known as chondromodulin-like proteins), arenicins (antimicrobial proteins from lugworms), and the group C proteins (also termed C16Orf79, chromosome 16 open reading frame 79). BRICHOS domain-containing proteins are linked to dementia (Bri2), respiratory disease (proSP-C), and cancer/tumor suppression (ChM-I and GKN2).[11,13−15] The family of BRICHOS domain-containing proteins also includes proteins with unknown function, several of which have never been studied at the protein level. Studies of BRICHOS domains have so far been limited; only the proSP-C and Bri2 BRICHOS domains have been studied in more detail, and a three-dimensional structure has been experimentally determined only for the proSP-C BRICHOS domain.[12] These studies suggest that the BRICHOS domain is involved in binding to β-prone regions during proprotein folding and processing.[16−18]

The proteins in the BRICHOS domain superfamily share a common overall architecture, are known or predicted to be type II transmembrane (TM) or secretory proteins, and have a conserved overall pattern.[11] All BRICHOS domain-containing proteins consist of a short cytosolic part, a hydrophobic, very likely TM domain, followed by, in the endoplasmic reticulum (ER) lumen, a linker region, the BRICHOS domain, and (in all cases except proSP-C) a C-terminal region. The BRICHOS domain is the only region of the BRICHOS domain-containing proteins that is conserved across the entire superfamily.[10] There are low or nonexistent levels of sequence similarity in the rest of the proteins. The BRICHOS domain shows very low levels (down to ~15%) of pairwise sequence identity between different families. As is generally the case,[19] fold is more conserved than sequence, and all regions display notable similarities in their predicted secondary structures.[10] The C-terminal regions can be proteolytically released, and in the case of proSP-C and Bri2, proteases are known to cleave at other sites, as well.[20,21] In almost all of the proteins, the C-terminal regions contain multiple Cys residues and at least two stretches with high β-sheet propensities interrupted by a short predicted coil region.[10,11] The only exception is proSP-C, which ends with the BRICHOS domain; proSP-C, on the other hand, has a TM region with exceptionally high β-sheet propensity.[22] All of the β-sheet-prone C-terminal regions of the BRICHOS proteins (the TM region for proSP-C) are well-conserved in their respective families.[10]

Directly after being identified, the BRICHOS domain was proposed to be involved in post-translational processing of the corresponding proproteins and/or to have a chaperone-like function.[11] This hypothesis is strengthened by experimental data, which indicate that the BRICHOS domain of proSP-C may work as a molecular chaperone that facilitates α-helical formation of the proSP-C TM region, thereby preventing amyloid formation.[12] Whether the interaction between the BRICHOS domain and TM region of proSP-C occurs intra- and/or intermolecularly is not known. It is likewise not known if additional, as yet unidentified, proteins are involved in the interactions of the BRICHOS domain with clients. The Bri2 BRICHOS domain has been shown to bind peptide Bri23, derived from the Bri2 C-terminal region.[18] This suggests that the BRICHOS domain, at least in these cases, may prevent misfolding and/or incorrect disulfide pairing, thereby preventing aggregation of β-sheet-prone regions in the respective precursor protein. Recent data suggest that this function may extend to Aβ and APP (see below).

The first crystal structure of a BRICHOS domain was recently determined and revealed a novel fold composed of a five-stranded β-sheet with an α-helix on each side (Figure 2).[12]

BRICHOS DOMAIN AND AMYLOID FIBRIL FORMATION

The BRICHOS domains of Bri2 and proSP-C were recently reported to be potent *in vitro* inhibitors of Aβ42 and Aβ40 aggregation. The BRICHOS domain was shown to prevent aggregation and fibril formation of Aβ in amounts far below stoichiometric amounts, and Aβ was kept in a monomeric unstructured state for an extended time in the presence of the BRICHOS domain (Figure 3).[23] In contrast to other protein inhibitors that are found to prevent Aβ aggregation by monomer binding and depletion from solution[24] or by inhibition of elongation,[25] the BRICHOS domain most likely interferes with nucleation events.[23] There are several examples of proteins that have been found to interfere with Aβ fibril formation or to interact with Aβ oligomers. The extracellular chaperone clusterin, also known as apolipoprotein J, for example, is upregulated in AD brain tissue, is localized to amyloid plaques, and forms long-lived complexes with Aβ40 oligomers.[26] Another class of proteins that interact with Aβ consists of amyloid-forming proteins. The cellular prion protein[27] and transthyretin[28] are reported to bind to Aβ oligomers and affect their toxicity. Transthyretin appears to

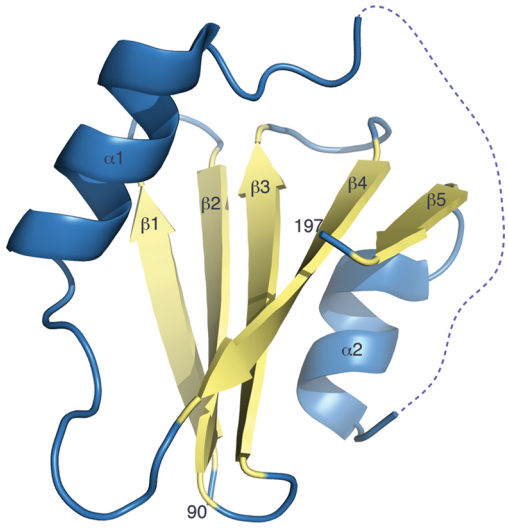

Figure 2. BRICHOS domain fold. Ribbon diagram of the proSP-C BRICHOS domain (PDB entry 2YAD) illustrating the fold of BRICHOS domains. The view is looking down onto face A of the β-sheet. Secondary structure elements are labeled. The numbers indicate the first and last residue in the structure. The dashed line indicates the disordered connection between helices α1 and α2.

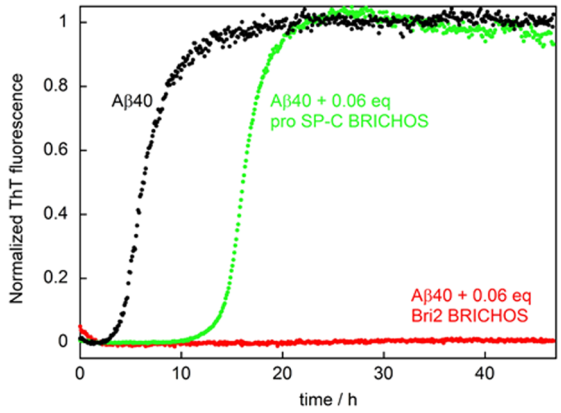

Figure 3. Effects of the BRICHOS domain on Aβ40 fibril formation. Fibril formation is measured by ThT fluorescence and shows a lag phase, an elongation phase, and a steady state plateau phase. In the presence of 6 mol % BRICHOS domain from proSP-C, the lag phase is prolonged, while in the presence of the same amount of the Bri2 BRICHOS domain, fibril formation is not observed. The Aβ40 concentration was 6 μM.

be particularly interesting because its dissociation from a tetramer to monomers exposes hydrophobic surfaces that can scavenge Aβ oligomers.[28]

The studies of the effects of the BRICHOS domain on Aβ fibril formation took advantage of a recently developed protocol for obtaining reproducible Aβ aggregation kinetics, which arises from multiple parallel processes in macroscopic samples.[29] Key considerations are Aβ expression in *Escherichia coli* to obtain homogeneous sequence, purification of highly pure peptide,[30] monomer isolation in degassed buffer, minimized and inert surfaces,[31] and optimized thioflavin T (ThT) concentration.[32] The shape of each aggregation curve is governed by the protein concentration, the rate constants for all underlying processes, and the starting state (pure monomer, monomer mixed with aggregates, or monomer mixed with the BRICHOS domain).

Amyloid fibril formation is a nucleated polymerization reaction that behaves like a phase transition.[31] Sigmoidal-like growth curves appear when aggregation is monitored as a function of time. After a lag phase, the emergence of new fibrils is a rapid process, followed by an equilibrium plateau (Figure 3). Recently, the aggregation mechanism for Aβ42[32] was found to include two kinds of nucleation. By the first kind, primary nucleation, monomers associate in solution and form a nucleus. By the second kind, surface-catalyzed secondary nucleation, monomers attach close to each other on the surface of already existing fibrils and form a nucleus that then detaches.[32] This secondary nucleation process forms an autocatalytic feedback loop. Moreover, oligomers generated by secondary nucleation are toxic.[32] Oligomers resulting from primary nucleation might also be toxic; however, this is inherently difficult to study because with the relative values of the rate constants for primary nucleation, elongation, and secondary nucleation, the first fibrils emerge very early during the lag phase, and from an early stage in the process, a majority of the oligomers arise due to secondary nucleation.[32] This motivates the search for compounds that interfere with secondary nucleation, and future studies will explore whether the BRICHOS domain has such activity.

■ ASSOCIATION OF THE BRICHOS DOMAIN WITH AMYLOID DISEASE

Patients with mutations located in the proSP-C BRICHOS domain develop interstitial lung disease (ILD), intracellular accumulation of aggregated proSP-C (or parts thereof), and reduced amounts of secreted mature SP-C.[33,34] Biochemical, cell culture, and *in vivo* data suggest that the BRICHOS domain of proSP-C is critical for the folding of the proSP-C TM part, which corresponds largely to the mature SP-C peptide, into an α-helix and thereby prevents it from forming amyloid. In patients with two different mutations in the proSP-C BRICHOS domain, we found evidence of amyloid deposits in the diseased lung tissue, and these deposits showed immunoreactivity specifically with antibodies toward the mature SP-C.[12] These findings motivate further research about the potential ability of BRICHOS domains to function as anti-amyloid chaperones.[35]

Familial British dementia (FBD) and familial Danish dementia (FDD) show similarities to AD with respect to symptoms, distribution of neurodegeneration, and the presence of amyloid deposits and neurofibrillary tangles.[36] FBD and FDD are caused by two different point mutations in the Bri2 gene, both of which give rise to a loss of the normal stop codon and C-terminal peptides, ABri and ADan, respectively, extended by 11 residues compared to the Bri23 peptide generated under normal conditions.[15,37] The Bri23 peptide binds to the Bri2 BRICHOS domain,[18] but it is not known whether the BRICHOS domain interacts with the ABri and ADan peptides. The ABri and ADan peptides form amyloid fibrils, which are deposited in the brain of FBD and FDD patients. However, it has been questioned whether ABri and ADan as such give rise to disease or if a loss of function of the Bri2 protein and concomitant effects on the processing of the AD-associated APP or the aggregation of Aβ also contributes.[38−40]

AD is a devastating disease with memory loss and personality changes, which is characterized histopathologically by extracellular senile plaques composed of Aβ and intracellular neurofibrillary tangles composed of tau.[41] The Aβ peptide is being generated in various lengths, most commonly, Aβ40 and Aβ42; Aβ42 is much more prone to aggregate and forms the most toxic species.[42] It has been shown that Bri2 interacts with

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and affects the processing of APP and that FDD patients have altered levels of APP metabolites, including increased levels of Aβ,[43] but it is not known exactly what part(s) of Bri2 mediates these effects. Both the Bri2 BRICHOS domain[18] and the Bri23 peptide[40] in isolation have been shown to reduce the extent of fibril formation of Aβ. It has also been shown that the anti-amyloidogenic effects of Bri2 in APP transgenic mice require the presence of the Bri23 peptide segment.[40] Bri3, another member of the Bri gene family, has also been shown to interact with APP, suggestively by blocking the access of the secretases to APP, thereby inhibiting Aβ production.[44] Recently, it was shown that transgenic mice overexpressing Aβ42 as a fusion protein with Bri2, where the normal C-terminal peptide Bri23 was substituted for Aβ42, were not cognitively affected even though they had high levels of Aβ42 expression and eventually developed amyloid plaques.[45] The authors suggested that high Aβ42 levels and aggregates are not sufficient to induce memory dysfunction and that APP processing derivatives are contributing to the toxicity seen in APP transgenic mouse models.[45] The Bri2 BRICHOS domain has previously been shown to be released from the proprotein by proteolysis,[21] and an alternative explanation of the lack of toxic effects seen in the Bri2- and Aβ42-expressing mouse model[45] could be that the BRICHOS domain delays Aβ aggregation and toxicity.

PROPERTIES OF POLYPEPTIDES THAT INTERACT WITH THE BRICHOS DOMAIN

In the absence of systematic studies of BRICHOS domain substrate specificity, insights into its molecular determinants can be gained from an overview of known target sequences for various BRICHOS domains. The recombinant proSP-C BRICHOS domain displays a micromolar affinity for unfolded full-length SP-C, for peptides covering the proSP-C TM part, and for oligo-valine peptides, but no binding has been observed to natively folded (helical) SP-C or to peptides with polar side chains.[16,46] Interestingly, the proSP-C BRICHOS domain binds specifically to nonpolar homodecapeptides with a high propensity for insertion into the ER membrane.[17] The substrate specificity of the proSP-C BRICHOS domain shows similarities, but also differences, with chaperones of the Hsp70 family, DnaK and BiP, which have affinity for a multitude of cellular substrates. The ER resident chaperone BiP recognizes seven-residue peptide segments with alternating patterns of hydrophobic residues, compatible with the prevalence of peptides with a high β-strand propensity found after selection from a phage-display library.[47] However, while Trp is the most favored residue in BiP substrates, the proSP-C BRICHOS domain has low affinity for poly-Trp. The central five residues in peptides with high affinity for DnaK[48] have a character similar to that of those conferring substrate specificity of the proSP-C BRICHOS domain. The affinity for DnaK is further enchanced by flanking basic residues, a property not observed for the proSP-C BRICHOS domain.

Although the primary structural properties of peptide substrates are similar for the BRICHOS domain and some general chaperones, the role of secondary structure may differ. On the basis of hydrogen−deuterium exchange mass spectrometry data, it was proposed that the proSP-C BRICHOS domain has a high affinity for a β-hairpin structure.[12] More experimental studies of BRICHOS domain client specificity with regard to secondary structure are warranted, but β-hairpin structures may be a target motif for several BRICHOS domains, because all regions C-terminal to the BRICHOS domain contain at least two stretches that are predicted to form β-strands. In the case of arenicin, NMR data show that the C-terminal region indeed forms a β-hairpin in solution.[49] The trefoil factor that binds to the GKN2 BRICHOS domain likewise forms a hairpin structure in solution,[50] and the GKN1 BRICHOS domain from chicken gizzard binds to a strand−turn−strand structure motif in filamentous actin.[51]

General chaperones like the Hsp's have very broad substrate specificities, with moderate or high affinity for almost any incompletely folded protein, and typically affect the aggregation of partly unfolded insulin or alcohol dehydrogenase, common substrates for testing general chaperone activity. The proSP-C BRICHOS domain lacks this function,[52] and although these two examples may not represent unfolded proteins in general, the BRICHOS domain from proSP-C seems to have a more restricted substrate specificity. Still, the proSP-C BRICHOS domain interacts with the amyloidogenic peptides Aβ and medin, which share no sequence similarities with each other or with SP-C.[52] Like SP-C, Aβ is produced from a membrane-spanning proprotein, APP. The Bri2 BRICHOS domain binds the Bri23 peptide and Aβ, but not nonpolar oligopeptides that are substrates of the proSP-C BRICHOS domain.[18]

HOMOLOGY MODELS OF BRICHOS DOMAINS

Mapping of conserved residues and ILD-associated point mutations on the structure of the BRICHOS domain of proSP-C led us to propose that face A of the BRICHOS domain β-sheet might be involved in target peptide binding.[12] Moreover, the side chains exposed at the proposed target peptide binding site on the BRICHOS domains of proSP-C and Bri2 appeared to correlate with the characteristics of the target peptides.[23] Whereas proSP-C is thought to interact with and act on the very hydrophobic SP-C and linker regions of proSP-C, the proposed Bri2 BRICHOS domain target peptide, i.e., the C-terminal Bri23 region, is much more polar and harbors many charged residues. Correspondingly, several hydrophobic residues on face A of the proSP-C BRICHOS domain are substituted with charged residues in Bri2.

To investigate whether a similar correlation could be observed for other families of BRICHOS domain-containing proteins, we first analyzed predicted and known target peptides from human BRICHOS protein families to identify conserved features (Table 1). Sequences from the known families of BRICHOS domain-containing proteins were collected from the NCBI/HomoloGene database, extended by a BLAST search to include sequences of additional family members. Truncated or fragment sequences were excluded as were those belonging to the previously identified group A,[10] because of significant variation of predicted target sequences within the group. This analysis shows that target peptides from different families vary greatly in the number of charges, as well as in the frequency of charged residues (charge density) and net charge (Table 1). For example, the very hydrophobic SP-C and group C peptides have a very low charge density compared to the others, while the Bri families, and in particular Bri1, have a high charge density. The target peptides from Bri, GKN2, and group C family proteins have low formal net charges, whereas SP-C, ChM-I, and TNMD have formal charges ranging from ±3 to ±5. GKN1 has a net negative charge of −4. Most, but not all, peptides have a higher proportion of residues with high β-sheet propensity (VCIF) compared to the average protein composition. All of the target peptides except the SP-C TM segment contain at least two conserved cysteine residues at positions compatible with the formation of hairpin-stabilizing disulfide bridges (Figure 4). The two cysteines in the Bri2 peptide are known to form a disulfide bond.[53] Given the

Table 1. BRICHOS Domain Consensus Target Sequences

Family	*Consensus target sequence*§	*% id*¶	*C*#	+£	-£	β£	α£	N±
Bri1	RAIDKCWKIRHFPNEFIVETKICQE +ab-+b +b++b -bbb- +bb -	28	+2	24 (6) 1.76	16 (4) 1.39	36 2.07	4 0.20	12
Bri2	REASNCxTIRHFENKFAVETLICS +-a b b++b- +bab- abb	42	+1	17 (4) 1.25	13 (3) 1.13	29 1.68	13 0.63	15
Bri3	RGAKNCNAIRHFENTFVVETLICGVV + a+ b ab++b- bbb- abb bb	58	+2	15 (4) 1.13	8 (2) 0.67	38 2.21	12 0.58	13
GKN1	MAEEIQGANLILYSEKCYTADILWILNISFCxxxVEN aa--b a aba -+b a-ba ba b bb b-	27	−4	3 (1) 0.22	14 (5) 1.22	24 1.38	22 1.12	9
GKN2	KGEVVENTHNVGAGxCAKAGLLGILGISICADIHV + -bb- + b a ba+a aa ba b bba-b+b	14	+1	11 (4) 0.81	9 (3) 0.78	29 1.67	20 1.02	9
ChM-I	SMTFDPRLDHEGICCIECRRSSYTHCQKICEPLGGYYPWPYNYQGCRSACRVIMPCSWWVARILGMV a b- +a-+- bbbb-b++ +b +bb- a b+ ab+bba b ba+ba ab	60	+4	13 (9) 0.99	7 (5) 0.65	25 1.46	12 0.61	14
TNMD	NGIEFDPMLDERGYCCIYCRRGNRYCRRVCEPLLG-YYPYPYCYQGGRVICRVIMPCNWWVARMLGRV b-b- aa--+ bbb b++ + b++bb- aa b +bbb+bba b ba+aa +b	69	+5	15 (10) 1.10	7 (5) 0.65	27 1.54	12 0.61	12
Group C	GPRRQRLIYLCIDICFPSNICVSVCFYYLPD ++ +ab abb-bbb bbb bbb a -	61	+1	10 (3) 0.71	6 (2) 0.56	39 2.22	10 0.49	13
proSP-C	HLKRLLIVVVVVVLVVVVIVGALIMGL +a++aabbbbbbbabbbbbb aaba a	48	+3	11 (3) 0.81	0 (0) 0.00	52 2.99	30 1.52	35

§The first line is the consensus amino acid sequence of predicted[60] BRICHOS domain target peptides in one-letter code with strictly conserved residues shown in bold. Conserved cysteine residues are highlighted. The second line shows residue type: +, positively charged residues (HRK); −, negatively charged residues (DE); b, VCIF; a, LAM. ¶Intrafamily sequence identity. #Net formal charge. £Residue frequencies (percent) of residue types HRK (+), DE (−), VCIF (β), and LAM (α) in the target peptides. Counts for charged residues are given in parentheses. Numbers below the frequencies are (observed frequency)/(average frequency in protein) (frequency in proteins from ref 61). ±Number of sequences.

high degree of cross-family similarity between the Bri target peptides, it appears likely that the two cysteines in Bri1 and Bri3 also form disulfide bonds. The gastrokines have a pair of cysteine residues with the same spacing toward the C-terminal part of the target sequence. It is possible that these cysteines, in analogy to the Bri case, form disulfide bonds.

To look for potential correlated features in the corresponding BRICHOS domains, we first conducted multiple-sequence alignment for each family individually, and for all sequences at once. Because of the low degree of sequence conservation between families (typically 12−30% identities for pairwise alignments), multiple-sequence alignment is not trivial, and care must be taken to avoid pitfalls. For example, initial attempts to align all of the sequences using Clustal Omega[54] failed to align the three residues known to be strictly conserved in all BRICHOS domains (Asp 105, Cys 121, and Cys 189 in proSP-C) while also introducing gaps in known secondary structural elements. However, using the state-of-the-art FFT-NS-i strategy in MAFFT,[55] a reasonable alignment for the part of the sequences corresponding to the known structure of the proSP-C BRICHOS domain could be achieved. Because the level of sequence conservation is particularly low in the region between the two helices of the domain (see Figure 2) and this segment also varies considerably in length, the alignment within this region remains unreliable.

On the basis of the MAFFT all-family alignment, we then constructed homology models for the human sequence from each family. The automodel module of MODELER[56] was used to generate homology models with the crystal structure of the proSP-C BRICHOS domain (PDB entry 2yad) as a template. All of the produced models have good internal side chain packing, showing that all of the predicted BRICHOS domains are compatible with the proSP-C BRICHOS domain fold. However, because of the generally low level of sequence conservation between families, we restricted further analysis to include only the location of side chains on the more conserved secondary structural elements, i.e., the five strands of the β-sheet that make up the core of the fold and are thought to be involved in target peptide binding, at least in proSP-C.

As for proSP-C, the central five-stranded β-sheet of the BRICHOS domain is the most highly conserved part of the structure in all of the BRICHOS protein families. Furthermore, as in the proSP-C family, in all of the examined sequences, there is a very strong tendency for aromatic residues on face A of the sheet at positions corresponding to proSP-C residues 104, 106, 113, 122, and 195 (Figure 4A). Interestingly, positions 104 and 106 bracket the strictly conserved Asp (Asp 105 in proSP-C), whereas residues at positions 113, 122, and 195 are located opposite the strictly conserved disulfide formed by Cys 121 and Cys 189. The conserved aromatic residues form a characteristic structural feature reminiscent of polyphenolic compounds known to retard Aβ aggregation[57] and have been suggested to play a role in binding and stabilizing β-strand regions of the target peptide.[23]

As previously noted, face A of the proSP-C BRICHOS domain consists almost entirely of hydrophobic residues, matching the hydrophobic SP-C target sequence. In contrast, faces A of BRICHOS domains in Bri proteins are significantly charged (Figure 4A). The proposed target sequences of Bri proteins contain a pair of conserved cysteine residues flanking a 16-residue sequence that harbors between four and six charged residues and two predicted β-strands (Table 1 and Figure 4B). Although it is not possible to formulate any detailed hypotheses regarding binding of Bri target sequences to Bri BRICHOS domains, it is plausible that the swap from the hydrophobic target and face A residues in proSP-C to charged ones in Bri proteins reflects their involvement in interactions between the two parts of the protein.

Similarly to the Bri proteins, the gastrokines form a supergroup with clear correlations between target sequences as well as BRICHOS domains. As in the Bri proteins, the target sequences have a pair of conserved cysteine residues at each end of two predicted β-strands. The gastrokine sequences between the cysteines are significantly less charged than in the Bri proteins. Correspondingly, face A of the gastrokine BRICHOS domains contains significantly fewer charged residues (Figure 4).

ChM-I and TNMD are homologous glycoproteins containing a BRICHOS domain followed first by a hydrophilic glycosylated region and then by a highly conserved (63% identity between the human proteins) cysteine rich hydrophobic domain.[58] Mature ChM-I is a 25 kDa protein produced from the proprotein by furin cleavage to release the two C-terminal domains. The cyclic

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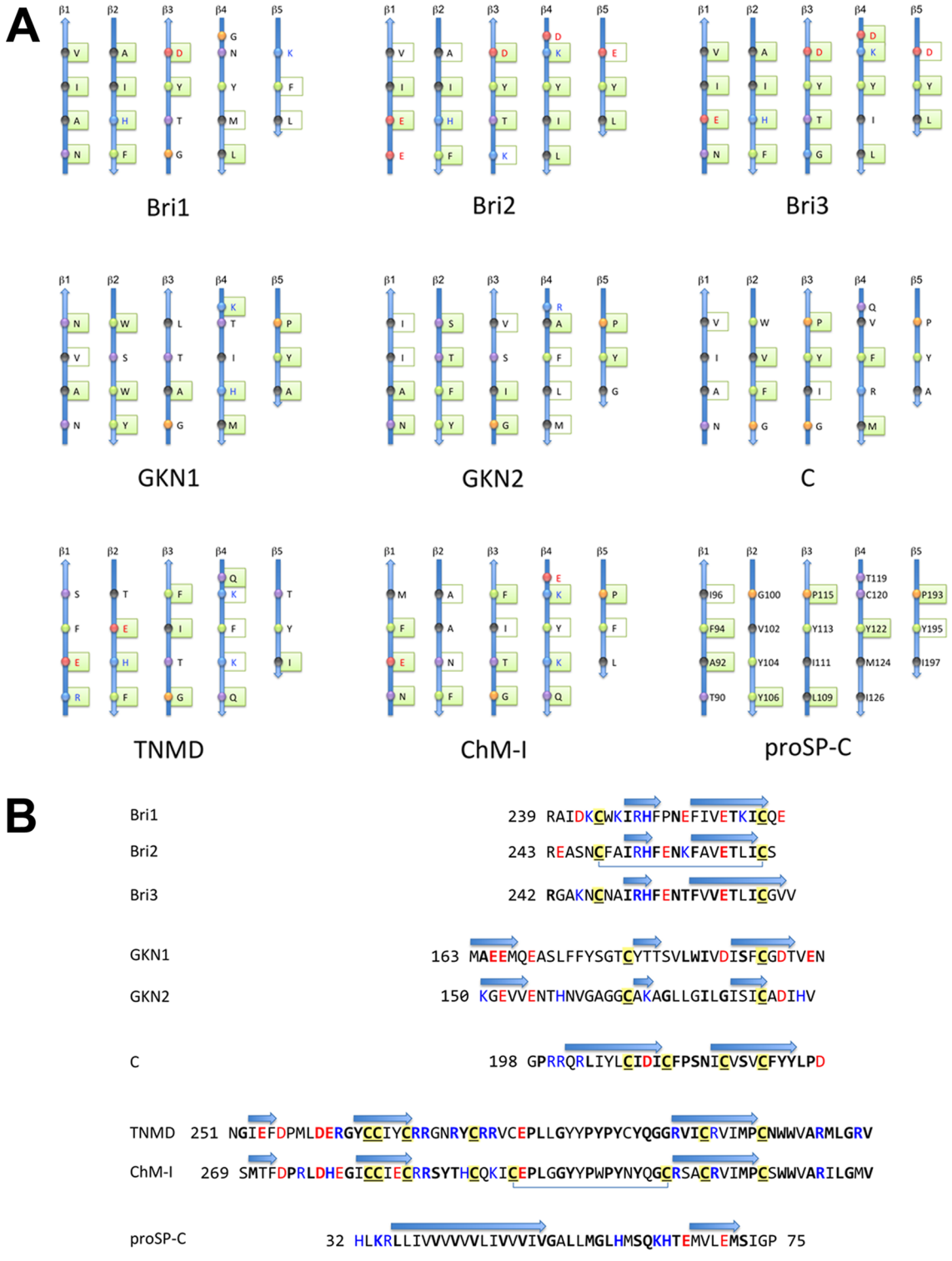

Figure 4. Face A and predicted target peptides of human BRICHOS domain-containing protein families. (A) Schematic representations of face A of the BRICHOS domain β-sheet from different families of BRICHOS domain-containing proteins. Residue positions where the residue character is conserved throughout the family are boxed, and positions that are identical within a family are boxed and shaded. Spheres representing residues are colored according to character: purple for polar, gray for nonpolar, green for aromatic, orange for Gly/Pro, red for negative, and blue for positive. (B) Human target peptides with predicted β-strands[10] indicated by arrows and strictly conserved residues shown in bold. Charged residues are colored red (negative) and blue (positive); cysteine residues are highlighted in yellow, and known disulfide bridges are indicated by connectors. In both panels A and B, residues are denoted by their one-letter amino acid code.

structure formed by the disulfide bridge between Cys 83 and Cys 99 in human ChM-I has been shown to be required for its anti-angiogenic function.[59] The involvement of the 10-residue hydrophobic tail at the C-terminus in ChM-I function has also been suggested. Because of the much larger size of the post-BRICHOS region in ChM-I and TNMD compared to Bri and GKN, it is difficult to speculate about interactions between these regions and the BRICHOS domains. However, it is interesting to

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note that the sequence between Cys 83 and Cys 99 contains a large number of aromatic residues that could potentially interact via aromatic stacking with the aromatic residues on face A of ChM-I and TNMD BRICHOS domains (Figure 4).

The C16orf79/group C family has not been studied experimentally at all but seems to be a particularly interesting target for such studies because it contains a C-terminal extension that is highly conserved from humans to fish.[10] There are four Cys residues in the group C C-terminal peptides, and the segment bracketed by the outer Cys pair is mainly hydrophobic. Correspondingly, face A of the group C BRICHOS domain contains almost exclusively hydrophobic residues (Figure 4).

CONCLUSIONS AND OUTLOOK

Studies of amyloid fibril formation of an unusual peptide, SP-C, initially suggested that the BRICHOS domain is one of nature's (more specific) ways to protect against fibril formation.

Homology models suggest a common binding face in BRICHOS domains from different families, which is complementary to their respective substrate peptides.

Bri2 and proSP-C BRICHOS domains can target also amyloid-forming peptides other than the ones present in the corresponding proprotein, in particular Aβ. Highly reproducible data for Aβ fibrillation kinetics can be used to generate a detailed mechanistic description of the action of the BRICHOS domain.

The efficiency, specific mechanism, small size, and natural occurrence suggest that the BRICHOS domain could be harnessed as a therapeutic against AD (and other amyloid diseases), by adding it exogenously and/or by activation of the endogenous protein. For AD, the Bri proteins seem to be particularly relevant as they are expressed in the same cells as APP.

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ABBREVIATIONS

Aβ, amyloid β-peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; ChM, chondromodulin; ER, endoplasmic reticulum; FBD, familial British dementia; FDD, familial Danish dementia; ILD, interstitial lung disease; GKN, gastrokine; PDB, Protein Data Bank; SP-C, surfactant protein C; ThT, thioflavin T; TM, transmembrane; TNMD, tenomodulin.

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