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Conserved Amphipathic Helices Near the N-Terminus and C-Terminus of the Alpha Subunit of Cranin (Dystroglycan)

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Cranin (dystroglycan) is a ubiquitously expressed extracellular matrix receptor, synthesized as a single precursor, which is cleaved into an extracellular subunit (alpha) and a transmembrane subunit (beta). The primary sequence of cranin (dystroglycan) is known from cDNA cloning, and the protein has been strongly implicated in morphogenesis, cell adhesion and human disease. Nevertheless, the domain structure of the alpha subunit has not been well studied; although the protein binds to matrix proteins, to the beta subunit, to cell surfaces, and possibly to other membrane proteins such as sarcoglycans, the domains responsible for mediating these interactions remain unknown. Here I report computer analyses that identify two distinctive amphipathic alpha-helical regions near the N-terminus and C-terminus of the alpha subunit, which are conserved in all species for which sequence information is currently available. This finding should stimulate and guide experimental studies designed to understand how the alpha subunit is associated with the cell surface and with its various ligands.

Keywords: Extracellular matrix, cranin, dystroglycan, mucin, GlyCAM-1, amphipathic, secondary structure

INTRODUCTION

Cranin (dystroglycan) is an ubiquitously expressed extracellular matrix receptor, synthesized as a single precursor, which is cleaved into an extracellular subunit (alpha) and a transmembrane subunit (beta) (Ibraghimov-Beskrovnaya *et al.*, 1992; review in Henry and Campbell, 1996). The alpha subunit is highly glycosylated and exhibits calcium-dependent binding to a set of matrix proteins including laminins, agrin, and perlecan; the beta subunit contains a cytoplasmic tail that binds directly to dystrophin and GRB2 (Henry and Campbell, 1996; Peng *et al.*, 1998).

The primary sequence of cranin (dystroglycan) is known from cDNA cloning (Ibraghimov-Beskrovnaya *et al.*, 1992), and the protein has been strongly implicated in morphogenesis, cell adhesion

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and human disease (e.g., Durbeej *et al.*, 1995; Belkin and Smalheiser, 1996; Henry and Campbell, 1996; Williamson *et al.*, 1997). Nevertheless, the domain structure of the alpha subunit has not been well studied; although the protein binds to matrix proteins, to the beta subunit, to cell surfaces, and possibly to other membrane proteins such as sarcoglycans, the domains responsible for mediating these interactions remain unknown. Several groups have proposed a model for the alpha subunit in which an N-terminal globular domain binds directly to matrix proteins, a central mucin-like domain acts to modulate the affinity of binding, and a C-terminal globular domain is responsible for direct binding to the beta subunit (Brancaccio *et al.*, 1995; Henry and Campbell, 1996). In contrast, I have pointed out that cranin shares a number of structural and ligand-binding similarities with sialoglycoproteins such as GlyCAM-1, and have proposed that specific oligosaccharide sequences within the mucin-like domain of cranin directly mediate binding to matrix proteins (Smalheiser, 1993; Smalheiser and Kim, 1995).

Here, I have explored the possible structural analogies between GlyCAM-1 and alpha-dystroglycan further. Noting that GlyCAM-1 and several other sialoglycoproteins (e.g., PP3 and densin-180) exhibit amphipathic helix domains that appear to be involved in their association with cell surfaces (Dowbenko *et al.*, 1993; Johnsen *et al.*, 1995; Apperson *et al.*, 1996), I carried out an analysis of sequences in search of amphipathic helices in alpha-dystroglycan. As documented here, two such domains were identified near the N-terminus and C-terminus, respectively.

METHODS

GenBank cDNA sequences for the alpha subunit of human, rabbit and mouse cranin (alpha-dystroglycan) were translated and analyzed using the Wisconsin GCG software package (version 8, Sept. 1994; Genetics Computer Group, 525 Science Drive, Madison, WI 53711). Two independent strategies were pursued: First, all regions were identified which were predicted to form uninterrupted alpha-helices of at least 18 amino acids in length (by the Garnier–Osguthorpe–Robson algorithm within the PEPTIDESTRUCTURE program). These were plotted on an alpha-helical projection. Second, the helical hydrophobic moment was determined for each residue (by the PEPPLOT program); stretches having values of ≥ 0.4 over at least 18 consecutive amino acids were plotted on an alpha-helical projection. Three alpha-helical regions reached criterion (38–60, 527–545, and 616–637) as did two regions of high helical hydrophobic moment (38–60 and 496–513).

RESULTS

Only 38–60 and 616–637 exhibited discrete hydrophobic faces when the sequences were plotted on a helical projection (Fig. 1); these and their flanking sequences were analyzed further. The maximal region including 38–60 that can support amphipathicity is 29–70, which would exhibit a face containing 13 hydrophobic amino acids (Fig. 1(b)). The other face consists of a mixture of nonpolar, polar and charged residues, as is typical for amphipathic helices of class G (Segrest *et al.*, 1992). Because the N-terminus of the processed protein is blocked, the position of signal peptide cleavage is not known with certainty; although some authors suggest that the first residue is His30 (Ibraghimov-Beskrovnaya *et al.*, 1992; Brancaccio *et al.*, 1997), the algorithm of Nielsen *et al.* (1997) predicts that the most likely cleavage site is between residues 27 and 28 (data not shown). Regardless, the amphipathic region would lie near or at the N-terminus.

Examination of larger sequences that include 616–637 revealed that the maximal region that can support an uninterrupted hydrophobic face is 610–637. However, since the preceding sequences have the highest hydrophobic moments of any sequences within dystroglycan, regions that overlap 610–637 were also examined. Surprisingly, there potentially

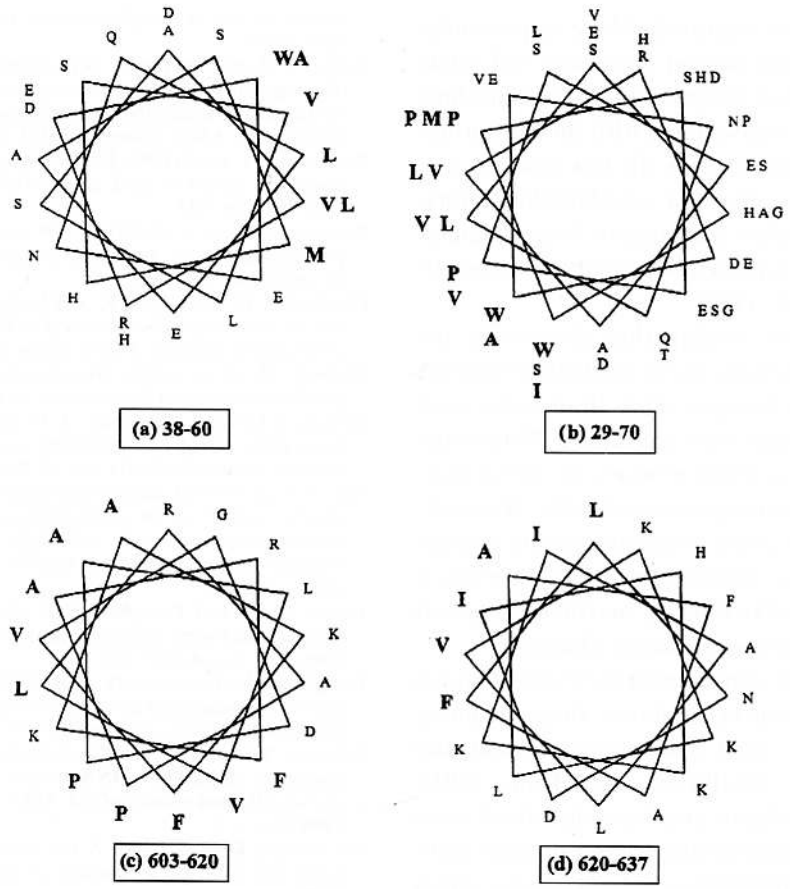

FIGURE 1 Alpha-helical projection of sequences derived from human cranin (dystroglycan).

exist *three* hydrophobic faces within the overall region 603–637: 603–620 supports a helix with *two* hydrophobic faces (Fig. 1(c)), and a third is supported in 620–637 (Fig. 1(d)).

DISCUSSION

Computer analyses were employed to identify two highly distinctive, discrete regions near the N-terminus and C-terminus of the alpha subunit of cranin (dystroglycan). These are likely to represent functionally important domains: (a) The sequence 38–60 was identified in two independent strategies as having both strong alpha-helical character and high average helical hydrophobic moment along its length; the entire region 29–70 potentially can form a single amphipathic helix. (b) The sequence 603–637 is even more unusual, in that it supports three hydrophobic faces (Fig. 1(c) and (d)). (c) It is noteworthy that the amphipathic character of 29–70 and 603–637 is strictly conserved across all species for which sequence information is available (man, rabbit, mouse and limited sequences available for Torpedo; data not shown).

The amphipathic character of these domains has not been noted previously, and indeed, previous analyses have not drawn attention to these regions at all. Their presence within the alpha subunit further supports our model of cranin (dystroglycan) as a mucin-like glycoprotein, and extends the list of mucin-like glycoproteins for which amphipathic

domains have been reported. More importantly, the present findings should stimulate and guide experimental studies designed to understand how the alpha subunit is associated with the cell surface and with its various ligands. In this context, it is worth mentioning that an amphipathic alpha-helical domain within the integrin beta-1 subunit has recently been reported to contribute to ligand-binding (Shih *et al.*, 1997).

The role of the amphipathic domain at the N-terminus is uncertain, but is unlikely to mediate binding to matrix proteins, since Brancaccio *et al.* (1997) demonstrated that the entire N-terminal globular domain covering residues 30–315 is incapable of binding matrix proteins directly. However, it is conceivable that the N-terminus could interact with the cell surface, leaving the oligosaccharides of the central mucin-like domain maximally exposed to facilitate recognition of matrix ligands.

The amphipathic domain near the C-terminus is a candidate to bind the beta subunit, thus explaining their tight noncovalent association (Ervasti and Campbell, 1993; Smalheiser and Kim, 1995). Although no candidate amphipathic helices were identified within beta-dystroglycan using the stringent criteria described above, several regions within the extracellular portion of the beta subunit do have high helical hydrophobic moment and exhibit hydrophobic faces when plotted on a helical projection: For example, 690–707 and 716–733 (not shown). Several laboratories have demonstrated that the alpha subunit can also be detected in a free, soluble, apparently full-length form (Gee *et al.*, 1993; Brancaccio *et al.*, 1995; Matsumura *et al.*, 1997), raising the possibility that the strength of the alpha–beta association may be subject to regulation, and that the free and the membrane-bound forms of the alpha subunit may have different degrees of exposure of the amphipathic domains.

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