Abstract

Testicans are modular proteoglycans of the vertebrate extracellular matrix with a broad tissue spectrum of expression. The protein family consists of three representatives, testican-1, -2, and -3, which are highly conserved. In their polypeptide chain, there are three regions homologous to other proteins, corresponding to follistatin-like domain (FS), extracellular calcium-binding domain (EC), and thyroglobulin domains (TY), while the N- and C-terminal regions are unique. They are involved in cell attachment, migration, and neurite outgrowth, among other functions, and studies suggests at least partial functional redundancy within the protein family. Although the specific functions and properties described so far (binding of calcium ions, inhibition of cysteine proteases, and membrane-type matrix metalloproteases) have been assigned to individual domains and regions, their spatial organization is unknown. To shed light on this, we further characterized testican-2 as a representative of the protein family, in terms of calcium ion binding and structure, and was related this to its influence on cell migration. First, using isothermal titration calorimetry and structural models of the calcium-binding domain (EC), we showed that only one of the two potential calcium binding sites is active and that it is always occupied at extracellular calcium concentrations. Binding to another potential EF2 motif is deactivated by substitution at one of the canonical coordination sites, where an amino acid residue with a smaller polar side group compared to EF1 is replaced by a residue with a large hydrophobic side group, and this applies to all three members of the protein family. Subsequently, we used disorder analysis to show that the N- and C-terminal regions of all three testicans are most likely structurally disordered. This is consistent with the results of low angle X-ray scattering (SAXS) analysis showing the structural disorder of the N-terminal unique region, while the FS–EC–TY domain region is structurally more ordered. This is also illustrated by a family of models generated based on experimental SAXS data and individual domain models, which indicate interdomain interactions within the FS–EC–TY triplet. Calcium binding contributes to structural stabilization and is reflected in the slightly larger compactness of the testican-2 molecule. Subsequently, using cell gap closure tests, we showed that the core domain triplet FS–EC–TY is associated with increased cell migration. The same was shown for testican-1, suggesting functional redundancy within this physiological role. Our results provide a first insight into the structural organization of testicans and thus provide a good basis for further research at the level of structure-function.

Key words: testican, SPOCK, calcium-binding, SAXS, cell migration