Molecular Dynamics Simulations of Peptides from the Central Domain of Smooth Muscle Caldesmon

Abstract

The central domain of smooth muscle caldesmon contains a highly charged region consisting of ten 13-residue repeats. Experimental evidence obtained from the intact protein and fragments thereof suggests that this entire region forms a single stretch of stable α-helix. We have carried out molecular dynamics simulations on peptides consisting of one, two and three repeats to examine the mechanism of α-helical stability of the central domain at the atomic level. All three peptides show high helical stability on the timescale of the MD simulations. Deviations from α-helical structure in all the simulations arise mainly from the formation of long stretches of π-helix. Interconversion between α-helical and π-helical conformations occurs through insertion of water molecules into α-helical hydrogen bonds and subsequent formation of reverse turns. The α-helical structure is stabilized by electrostatic interactions (salt bridges) between oppositely charged sidechains with i,i+4 spacings, while the π-helix is stabilized by i,i+5 salt bridge interactions. Possible i,i+3 salt bridges are of minor importance. There is a strong preference for salt bridges with a Glu residue N-terminal to a basic sidechain as compared to the opposite orientation. In the double and triple repeat peptides, strong i,i+4 salt bridges exist between the last Glu residue of one repeat and the first Lys residue of the next. This demonstrates a relationship between the repetitive nature of the central domain sequence and its ability to form very long stretches of α-helical structure.

Key words: α-helix stabilization, Salt bridges, π-helix.

Introduction

Caldesmon is a ubiquitous protein component of the contractile machinery in both smooth muscle and non-muscle cells. The smooth muscle form can be divided roughly into three domains: the N-terminal domain (residues 1-250) containing a myosin-binding site, the central domain (residues 250-400), and the C-terminal domain (residues 400-756), which contains sites for actin and calmodulin binding and ATPase inhibition (1). While its precise physiological function remains undetermined, caldesmon appears to have a number of different activities in the regulation of thin filaments in response to a cellular calcium influx. For example, it has been shown that caldesmon can inhibit actomyosin-activated ATP hydrolysis in vitro (2-4) and bind to myosin (5-7). In addition, caldesmon has also been shown to interact with the calcium-bound form of the regulatory protein calmodulin (2, 3, 8). The central domain of smooth muscle caldesmon contains ten highly charged, 13-residue repeats and is believed to be responsible for the extended structure of the protein observed in EM images (9). Similar studies on a 285-residue proteolytic fragment (residues 166-450) of smooth muscle caldesmon have also been performed (1). The results, when combined with CD data, secondary structure prediction and hydrodynamic measurements (1, 10, 11) provide strong evidence that the repeating region of the central domain forms a single stretch of α-helix in solution which is stable with respect to variations in both temperature and pH (1). CD studies on synthetic peptides corresponding to segments of this region indicate that the α-helical
The potential helix-forming abilities of the smooth muscle caldesmon central domain deserves further study for a number of reasons: firstly, the presence of such a long helical domain probably has some important role in the function of the protein. Certainly it may serve as a flexible separator of the two domains, thereby allowing the N-terminal domain to contact myosin while the C-terminal domain is anchored to actin or other targets (1). In a more general sense, the presence of such a long, stable \( \alpha \)-helix represents a novel structural entity. Lengths of similar, solvent-exposed helical structures found in other proteins (for example, calmodulin (13)) are significantly shorter. The inherent \( \alpha \)-helical propensity of specific peptide and protein sequences is relevant to the study of early steps in protein folding involving helix formation. In particular, the peptides examined here are excellent models for the study of helix-stabilizing electrostatic interactions between sidechains.

In addition to the structural information provided by NMR, CD, and other experimental methods, molecular dynamics simulations of peptides in solution are useful because they can provide detailed information on these systems at an atomic level. MD simulations on isolated peptides derived from protein structures has become a popular method for the microscopic analysis of dynamics and energetics of these structural elements within the intact protein. This approach is particularly justified for the peptides studied here, as the central domain of caldesmon forms a single extended \( \alpha \)-helix that is stable in the absence of any tertiary interactions with either of the terminal domains. Moreover, the size and highly repetitive nature of the caldesmon central domain leads to significant overlap in NMR spectra, making this a difficult system to study experimentally.

**Methods and Materials**

We have chosen the 13-residue sequence KAEEERKAEEERA as a model sequence, as it occurs in a sequential triple repeat in the central domain of turkey gizzard smooth muscle caldesmon in a region of particularly high \( \alpha \)-helical content as determined by secondary structure prediction (1). In all of the simulations the N-termini of the peptides were acetylated and the C-termini amidated, in order to minimize end effects and thus more closely model the single, extended helix proposed for the central domain structure.

As mentioned in the *Introduction*, we have carried out separate MD simulations (with identical parameters) for single, double and triple repeats of the model sequence described above. All simulations were carried out using the Gromacs software package (14) version 2.0 and the GROMOS force field (15) with the modifications of van Buuren *et al.* (16). For the starting peptide configurations, the InsightII package version 98.0 (Accelrys, Inc.) was used to build the sequence into a right-handed \( \alpha \)-helix using \( \phi,\psi \) angle values of -65\(^\circ\), -40\(^\circ\), respectively. Following this, the single, double and triple repeat peptides were centered in cubic boxes of dimension 5 nm\(^3\), 10 nm\(^3\), and 15 nm\(^3\), respectively. As the Particle Mesh Ewald (PME) method (17, 18) was used for the calculation of electrostatic interactions, the box sizes were chosen so as to help minimize artifacts arising from the periodic boundary conditions (19) yet keep the computational expense reasonable.

The peptides were solvated by filling the boxes with multiple equilibrated configurations of 216 SPC water molecules (20), then removing any waters whose atoms approached any atom of the peptide to a distance less than the sum of their respective van der Waals radii. One randomly selected water molecule in each system was replaced with a sodium ion to achieve overall electroneutrality. The final simulation systems contained a total of 5910, 20526, and 49392 atoms for the single, double and triple repeats, respectively. The solvated configurations were then energy min-

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imized using the steepest descent method and a convergence tolerance of 0.001 kJ mol\(^{-1}\). The minimized configurations then underwent 1 nanosecond (ns) each of restrained dynamics in which an isotropic force constant of 1000 kJ mol\(^{-1}\) nm\(^{-1}\) was applied to all peptide atoms. This helped to ensure relaxation of the solvent. The temperature and pressure of the system were kept constant by weak coupling to temperature and pressure baths of 300 K and 1 bar using coupling constants of 0.1 and 1.0 ps, respectively. The resulting configurations were used for production runs of 100, 65 and 20 ns for the single, double and triple repeats, respectively. A time step of 2 fs was employed for all simulations. Coupling parameters for temperature and pressure in the production runs were identical to those used in the restrained dynamics. In all cases the peptide, solvent and ions were coupled to the temperature and pressure baths separately. The centre of mass motion of the entire simulation system was removed every step to maintain the effective simulation temperature at 300 K. PME was applied using a real-space cutoff of 0.9 nm, a Fourier spacing of 0.12 nm, and B-spline interpolation to fourth order. The LINCS procedure (22) was employed to restrain all covalent bonds to their equilibrium values.

**Results**

**Secondary Structure**

Figure 1 shows the secondary structure of the single repeat peptide as a function of simulation time. The initial \(\alpha\)-helical conformation remains stable for the first 16.5 ns of the simulation, with the notable exception of a few residues near the C-terminus which sample bend, turn and coil conformations after the first nanosecond. This is due to the inherently greater flexibility of residues near the helix termini. The comparatively greater loss of helical structure near the C-terminus as compared to the N-terminus is consistent with the observed tendency of \(\alpha\)-helices to unwind at their C-terminal ends (23). At 16.5 ns nearly the entire peptide assumes a \(\pi\)-helical conformation interspersed with some transient turns of \(\alpha\)-helix. At the point of transition from \(\alpha\)-helix to \(\pi\)-helix, a number of residues near the end of the helical structure assume turn conformations. These turn conformations also accompany the reversion back to \(\alpha\)-helical structure, which takes place at 18.5 ns. The relatively rapid transition back to an \(\alpha\)-helix and the apparent stability of this conformation during the ensuing 10 ns is consistent with the high \(\alpha\)-helical propensity of the peptide observed experimentally. Between 29 and 100 ns, however, the peptide continues to alternate in a similar fashion between \(\alpha\)-helical and \(\pi\)-helical structures.

Figure 2 shows the same information for the double repeat peptide. Strikingly, the \(\alpha\)-helical structure of the double repeat remains stable for almost the entire 65 ns of the simulation. As with the single repeat, loss of \(\alpha\)-helical structure is initiated at the C-terminus, but is significantly shorter-lived. As in the simulation of the sin-
Due to the much larger simulation system required, a simulation of only 20 ns was feasible for the triple repeat peptide. However, as can be seen from Figure 3, a significant α-helix to π-helix conversion also occurs in this case. Unlike the single and double repeat peptides, the C-terminal residues of the triple repeat remain in mainly α-helical conformations for the first 18 ns of the simulation, and turn formation begins at the N-terminus and middle of the sequence. For the last 2 ns of the simulation, nearly the entire peptide consists of a single, large π-helix. In contrast to the single repeat, in which “all or none” interconversions between α-helix and π-helix were common, the length of the triple repeat peptide results in a transition that gradually extends along the chain. Both α- and π-helical turns co-exist within the sequence at several points during the last 2 ns of the simulation.
In an earlier simulation, insertion of water into α-helical hydrogen bonds was identified as a possible route to π-helical structures (24). This and the prevalence of turn conformations prior to π-helix formation in our simulations may have particular relevance to the α- to π-helix transition mechanism: based on an analysis of helical segments in 35 protein crystal structures (25), reverse turns resulting from the insertion of water into helical hydrogen bonds have been implicated as helix folding/unfolding intermediates. Panel A of Figure 4 shows an analysis of hydrogen bonding distances from the original study on crystal structures: taking instances in which water molecules interacted with helices, the authors ranked these examples according to increasing distance between the oxygen water and the amide nitrogen (d3) of the relevant α-helical hydrogen bond. It was found that the distance between the water oxygen and the carbonyl oxygen (d1) involved in the same hydrogen bond remained fairly constant. However, below a certain value of d3, the distance between the carbonyl oxygen and amide nitrogen of the helical hydrogen bond (d2) undergoes a sharp transition to large values, indicating disruption of the helical hydrogen bond. Schematics of typical configurations for the respective distances are shown above the graph. Panel B shows an identical analysis from the single repeat simulation, where all instances of water insertion into any α-helical hydrogen bond...
bond were recorded. The distances involved were then ranked as in the earlier study (25). A rough agreement can be clearly seen: the distance between the peptide hydrogen bonds (d2) in the simulation increases as the water oxygen approaches the nitrogen atom, although the transition is not nearly as sharp as in the crystal structures. Also, the transition in d2 begins at a much larger value of d3 (4.5 Å) in the simulation compared to that in the crystal structures (3.4 Å). While the value of d1 stays comparatively flat in the simulations, there is also a small increase observed near small values of d3. The increasing value of d1 concomitant with larger values of d3 corresponds to the water molecule being far away from both groups.

Figure 5 shows the number of residues involved in α-helical, π-helical or turn conformations for the entire single repeat peptide during each of the insertion events, with the same ordering as in Figure 4. There is no direct hydrogen bond distance information contained in the graph: the distances discussed below were obtained from comparing the insertion event number on the abscissa with the relevant distance data in Figure 4B. Clearly, the relative amount of π-helix conformations gradually rises as water insertion and the disruption of α-helical hydrogen bonds proceeds, with a small peak at around d3 = 4 Å. This is slightly below the value corresponding to the start of the transition state from the simulation (4.5 Å). Correspondingly, the amount of α-helical structure makes a significant dip at this point. That these changes are involved with turn formation is seen in the values for that structure type, which start to increase just before the peak in the π-helical structure values. This increase continues with the degree of water insertion until very small values of d3 are reached, when turn content decreases sharply. At the same time, the relative amounts of α-helical and π-helical conformations increase and decrease, respectively, with α-helical conformations once again in the majority. This is actually in accord with the earlier study: conformations of residues having “water inserted” peptide hydrogen bonds (e.g., the left most example above the graphs in 4A) were found to be more like α-helical conformations than reverse turns or other observed conformations (25).

Helix-stabilizing Sidechain Interactions

As mentioned earlier, it is hypothesized that the stability of α-helical structure in the caldesmon central domain is due to the formation of many favorable electrostatic interactions between sidechains spaced i,i+4 in the sequence (12). Studies with suitably designed, unrelated model peptides have shown that oppositely charged sidechains spaced three residues apart (i,i+3) can also have a significant effect on α-helical stability. Interactions between positions i,i+2 and i,i+5 have little to no effect (26, 27). Also, complex salt bridges or “triads” can be formed between suitable sidechains at three consecutive i,i+4 positions. These salt bridge networks have a cooperative stabilizing effect, with the favorable free energy of an i,i+4 triad being 1 kcal/mol greater than the sum of the individual pairs (26). Finally, the effect of position within the sequence needs to be considered: experimental evidence shows that salt bridges with the acidic residue in an N-terminal position relative to the basic one are more stabilizing than those of opposite orientation (27-30).

The helical wheel diagram of Figure 6 shows that many possible salt bridges can exist in helical conformations of the peptides studied here. Within each repeat, there is the potential for four i,i+4 and four i,i+3 salt bridges. The repetitive nature of the caldesmon central domain may also contribute to helical stability: with the addition of repeats, sidechains of residues within one sequence unit can interact with those of the next, resulting in two additional i,i+4 salt bridges and one additional i,i+3 salt bridge per additional repeat, respectively. For both i,i+4 and i,i+3 salt bridges, examples of acid-base and base-acid orientations with respect to the N- and C-termini exist.

While i,i+5 sidechain interactions may not contribute significantly to α-helical stability, residues in this position are ideally situated for the stabilization of π-helical
structure and are therefore relevant for the analysis of our simulations. One possible i,i+5 salt bridge exists within each repeat (Arg6-Glu11 and analogous pairs). For each repeat added, an additional i,i+5 interaction becomes possible (Arg12-Glu17 and analogous pairs).

Finally, within each peptide there exists the possibility of 1 complex salt bridge or triad involving 3 consecutive residues with i,i+4 spacing (Glu3-Lys7-Glu11 and the analogous residues in consecutive repeats). Another potential triad is gained with each additional repeat (Glu10-Lys14-Glu18 and their analogs).

### i,i+4 Sidechain Pairs

In the single, double and triple repeats, there is the potential for the formation of 4, 10 and 16 i,i+4 stabilizing salt bridges, respectively. Figures 7 and 8 show the distance between the charged groups of residues with i,i+4 spacings within each repeat for the single and double peptide, respectively.

From Figure 7 it is apparent that not all of the sidechain pairs remain in close interaction throughout the simulation. From visual inspection, interactions between the 2 sidechain pairs nearer the N-terminus (Lys1-Glu5 and Glu3-Lys7, respectively) are the most stable, fluctuating near a distance of 0.5 nm for most of the simulation. The large distances between the C-terminal sidechain pairs, particularly Lys7-Glu11, are consistent with the comparatively lower helical structure content at this end of the peptide throughout the simulation. The large increase in the distance between Arg6 and Glu10 near 15 ns correlates well with the first $\alpha$- to $\pi$-helix transition in the simulation. The distance between Lys7 and Glu11 increases greatly following the breakdown of $\alpha$-helical structure near the 29 ns mark and the salt bridge does not reform again for the remainder of the simulation. Figure 8 shows the same information for the double repeat simulation. Graphs in the left column show the distance between i,i+4 sidechain pairs within the first repeat of the sequence, and those on the right the distance between the analogous pairs of the
second repeat. Overall, the fluctuations in the distances for most of the pairs are smaller than those of the least stable salt bridges (Arg6-Glu10 and Lys7-Glu11) of the single repeat. The exceptions to this are the Arg19-Glu23 and Lys29-Glu24 pairs near the N-terminus, which move apart as a result of turn and \(\pi\)-helix formation near the C-terminus of the peptide shortly after 43 ns. While the Lys1-Glu5 distance also increases during this time, these residues remain in an \(\alpha\)-helical conformation (see Figure 2). Although the fluctuations in the distances are smaller than in the 13-residue peptide, in many cases (particularly Arg6-Glu10), the average distance between many of the sidechain charge groups is greater than the average of the corresponding pairs in the single repeat.

By choosing a distance criterion for intact and broken salt bridges and counting the fraction of time each sidechain pair spends in each of these states, we can gain an idea of the relative strength of different salt bridges within each peptide during the simulations. To this end, we have chosen a cutoff of 0.5 nm for the formation of a salt bridge and calculated the ratio between configurations with intact and broken salt bridges for each sidechain pair. Applied to the single repeat, the ordering of the ratios for \(i,i+4\) sidechain pairs is as follows: Glu3-Lys7 (0.72) > Lys1-Glu4 (0.44) > Arg6-Glu10 (0.30) > Lys8-Glu12 (0.07). It is interesting to note that out of these four possible salt bridges the only sidechain pair with an N-C acid/base orientation, Glu3-Lys7, forms the strongest salt bridge.

The Glu-Lys pairs in the double repeat peptide also form the 3 strongest interactions in the order Glu10-Lys14 (1.32) > Glu16-Lys20 (1.28) > Glu3-Lys7 (0.50). The strongest interaction overall is that of Glu10-Lys14, which spans the boundary between the two sequence repeats. The other potential \(i,i+4\) salt bridge pair arising from the repetition of the sequence, Arg12-Glu16, is much weaker with a ratio of 0.12. The fact that strong salt bridge-forming ability seems limited to sidechain pairs with the negatively charged member nearer the N-terminus is consistent with the experimental results obtained for model peptides (27-30).

Analogous sidechain pair distance plots for the triple repeat simulation reveals much the same picture as that of the double repeat peptide within the timescale of the 20 ns simulation (data not shown). As judged by the distance criterion, 5 stable salt bridges exist in the triple repeat simulation: Glu24-Lys28 (3.33) > Glu16-Lys20 (2.82) > Glu10-Lys14 (2.37) > Glu30-Lys34 (2.14) > Lys1-Glu5 (1.25). Once again, Glu-Lys sidechain pairs form the closest interactions in the peptide. Again, salt bridges which span the separate repeats of the model sequence, like Glu10-Lys14 and Glu24-Lys28, are amongst the most stable.

\(i,i+3\) Sidechain Pairs

Formed/broken salt bridge ratios calculated in the same manner for \(i,i+3\) sidechain pairs revealed that interactions between these residues are much less frequent and shorter-lived in all three simulations (data not shown). This is in agreement with experimental results on sidechain pairs at different positions in model peptides (28). No evidence was found for a preference of an N-C acid/base orientation amongst the \(i,i+3\) sidechain pairs. In fact, in all three peptides the strongest interaction was observed for Lys1-Glu4, with ratios of 0.33, 0.368, and 1.093 for the single, double and triple repeats, respectively. In contrast to the \(i,i+4\) sidechain pairs, \(i,i+3\) pairs that bridged the individual sequence units were no more stable than other pairs on average.

Salt Bridge Triads

There is one possible Glu-Lys-Glu salt bridge triad for every repeat in the peptides studied here. In order to determine if there is any cooperativity in triad formation in the simulations, the distance criterion was used to count the fraction of time in which both constituent sidechain pairs formed an intact salt bridge, were too far
apart to interact appreciably, or only one of the pairs formed a salt bridge. While salt bridge triads did form periodically, no evidence of correlation (or anti-correlation) in the formation of salt bridge triads was found (data not shown). The fraction of the time in which the Glu-Lys sidechain pair formed a salt bridge was in all cases much greater than that of the neighboring Lys-Glu pair, again reflecting the greater stability of sidechain pairs with the former orientation.

**i,i+5 Sidechain Pairs**

In light of the transient π-helix formation observed in the simulations, it may be possible that these structures are stabilized by interactions between oppositely charged sidechains spaced i,i+5 along the sequence. Indeed, one pair of this type exists within each repeat (Arg7-Glu12 and its analogs), with 1 additional pair for each repeat added (Arg12-Glu17 and analogs), giving totals of 1, 3, and 5 for the single, double and triple repeat peptides, respectively. Examples of four representative i,i+5 salt bridges taken from the triple repeat simulation are shown in Figure 9.

Looking at the top plot of the figure and progressing downwards, one can see the behaviour of the i,i+5 salt bridges starting near the N-terminus and proceeding towards the C-terminus. Salt bridge formation occurs first in the most N-terminal sidechain pair (Arg12-Glu17). After this salt bridge has become stable, the next sidechain pair along the peptide (Arg19-Glu24) also forms a salt bridge, and so on. This mirrors the gradual α- to π-helix transition observed in the simulation, which proceeds from the N- to the C-terminus (compare with Figure 3). A similar phenomenon was observed for the (Arg19-Glu24) i,i+5 salt bridge in the double repeat peptide during the formation of π-helix near the C-terminus near 45 ns, while correlations between i,i+5 salt bridges and π-helix in the single repeat peptide were difficult to observe given the fact that these i,i+5 pairs remain in fairly close proximity in both α- and π-helices (data not shown).

**Discussion and Conclusions**

The results of the MD simulations on the single, double and triple repeats reflect the high α-helical propensity of the caldesmon central domain sequence: in the single repeat simulation, the peptide consistently returns to α-helical conformations following transitions to π-helical structures. In the double repeat simulation, a 26-residue helix remains remarkably stable for almost the entire 65 ns trajectory, with only one major structural alteration. Even in the case of the triple repeat, α-helical turns coexist with π-helical ones along the peptide chain. It is reasonable to guess that a return to larger stretches of α-helix would be likely in a somewhat longer simulation of the latter peptide.

Due to limitations imposed on the computational expense of the simulations by system size, it was necessary to carry out shorter simulations for the longer peptides. In a theoretical situation where complete conformational sampling for each peptide was possible, we would be able to rationalize their different behaviour (i.e., the varying extent of π-helical conformations adopted). As it stands, there may be a reason for the greater α-helical stability of the double repeat peptide compared to the others: statistical mechanical theories of the helix-coil transition (31) show that for short to medium length peptides, the balance of favourable enthalpic (hydrogen bond formation) and unfavourable entropic (restricting residues to an α-helical conformation) results in an “all-or-none” conversion between coil and helix. For longer peptides, however, a combinatorial entropy can arise from the different ways in which localized stretches of coil and helix can be distributed along the chain. Assuming that the α- to π-helix transitions observed in our simulations are similar to (or represent the initial stages of) a helix to coil transition, then this feature of helix-coil theory may help in interpreting the results. The conversion between α- and π-helical forms in the single repeat peptide may accurately be described as “all-
or-none”, while separate, localized stretches of $\alpha$- and $\pi$-helix can be observed along the triple repeat peptide at times in that simulation. This latter phenomenon may be a manifestation of combinatorial entropy. The double repeat peptide, with its apparently higher $\alpha$-helical stability, may represent an intermediate situation in which the length of the peptide is ideal for maintaining a single stretch of $\alpha$-helix.

While $\pi$-helical conformations have been observed in many recent MD simulations of peptides (24, 32, 33), it has recently been shown that this can be a result of deficiencies present in all the most commonly employed force fields (34). An analysis of $\phi, \psi$ angles from our simulations reveal that the $\pi$-helical conformations observed populate a region of conformational space which is artificially favored by the force field. However, our simulations are quite long by current standards, and allow for significant unfolding transitions to occur. Since the conversion of an $\alpha$-helix to a $\pi$-helix represents a rather modest structural transition, we consider the interconversion between $\alpha$- and $\pi$-helical structures to be a result of the helical stability inherent in the central domain sequence of caldesmon. In our particular case, the artificially frequent observation of $\pi$-helical conformations may have been exacerbated by the formation of $i,i+5$ salt bridges, further stabilizing these structures.

From an analysis of water-inserted helical hydrogen bonds in protein crystal structures, plausible unfolding pathways and mechanism for the $\alpha$-helix have been proposed (25). We have shown that water insertion events in the simulation of the single repeat are similar to that observed in crystal structures and that the formation of $\pi$-helical conformations (via turns) is correlated to this phenomenon. While deficiencies in current force fields render the proposition of $\pi$-helices as $\alpha$-helical unfolding intermediates questionable, it is still possible that this phenomenon occurs on timescales too short to be accessible by experiment. In the case of the peptides studied here, it is likely that the stabilizing sidechain salt bridges overcome the disruptive influence of water insertion into $\alpha$-helical hydrogen bonds, preventing further unfolding to coil conformations.

Analysis of electrostatic interactions between sidechains spaced $i,i+4$ reveal a strong preference for Glu-Lys salt bridges over those of opposite orientation. Similar results have been found experimentally (27-30) and are thought to arise from the favorable effect of the negative sidechain charge with the overall helix dipole. Of particular relevance to the $\alpha$-helical stability of the smooth muscle caldesmon central domain is the finding that strong salt bridges of this type exist across separate repeats. This provides a physical explanation of how the repetitive nature of the central domain sequence leads to such remarkable $\alpha$-helical propensity, and agrees with the experimental work of Wang et al. (12). Glu-Lys salt bridges are so heavily favored in our simulations that they may out-compete neighboring Glu residues 4 positions down the sequence from forming complex Glu-Lys-Glu salt bridge triads. While these triads do occur transiently in our simulations, there seems to be no cooperativity in their formation.

In summary, we have found that the central domain of caldesmon is rather flexible but remains in “unkinked” helical conformations, consistent with experimental evidence for a single helical conformation in this domain. The competition between $\alpha$-helical and $\pi$-helical structures in the central domain of smooth muscle caldesmon would enable it to act as a flexible linker through variation of its length without a complete loss of secondary structure. Both types of helical structure are potentially stabilized by electrostatic interactions between sidechains with suitable placement along the sequence. Such interactions, when acting between sequential repeats, may be key to the formation of very long helical structures observed for the central domain and provide a rationale for the evolution of such a highly charged, repetitive sequence.
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References and Footnotes


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