Two identical hydrophobic clusters are present on the same actin monomer: interaction between one myosin subfragment-1 and two actin monomers

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Abstract Two-dimensional hydrophobic clusters analysis (HCA) was used to compare the distribution of hydrophobic clusters along various actin sequence. HCA-deduced patterns were not altered by amino-acid variations throughout the evolution of actin and we observed similar hydrophobic motifs comprising myosin subfragment-1 ATP-independent binding sites. HCA suggested the presence of two groups of identical hydrophobic motifs (A_1 and A_2) which bound on each side of the S1 (63 kDa–31 kDa) connecting segment in relation with two actin monomers. This connection is important in communications between actin- and nucleotide-binding sites. We postulate that some relation and message between the two motifs A_1 and A_2 take place through myosin subfragment-1 (63 kDa–31 kDa) connecting segment.

Key words: Acto-myosin; Hydrophobic cluster; Structure comparison

1. Introduction

Identification of the actin–myosin interface is essential in understanding the cyclical enzymatic and mechanical process of myofibrillar contraction.

Atomic resolution of the actin structure [1,2] and the three-dimensional atomic model of F-actin decorated with rabbit chymotryptic-S1 [3] or *Dictyostelium* myosin S1 [4] revealed locations of the myosin head on F-actin. This molecular model of the actin–myosin complex derived from the X-ray structure reveals a close contact between a myosin subfragment-1 and two actin monomers. A first contact includes carboxyl residues 1, 2, 3, 4, 24, 25, 99 and 100. In a second contact, exposed actin hydrophobic residues 144, 341, 345, 349 and 352 are concerned. The third contact involves proline residues (332–333) of actin. All of these contacts involve a single monomer. A second monomer is close to the myosin heavy chain and the interaction includes the α-helix formed by actin residues Trp79 to Asn92 that formed a weak binding contact and was cross-linked [5,6].

The Taylor-Amos model [7] of acto-S1 suggests that S1 has an extensive contact with an actin monomer (ac1) and a weaker contact with an adjacent actin monomer (ac2); and two different rigor complexes were suggested [8,9], with S1 binding to F-actin occurring in two steps with actin monomers. Using successively EDC and DMS, we previously cross-linked two monomers to 20-kDa and 50-kDa skeletal S1 fragments, respectively [10], and in vitro studies showed that subfragment-1 alone can produce the sliding movement of the actin filament [11].

Chemical cross-linking [12], NMR [13,14] and immunochemical studies [15,16,17–20] have pinpointed actin segments in subdomain-1, residues 1–28, 96–103, 112–125, 338–348 and 360–372 which are able to bind with the myosin head.

Furthermore, the (27 kDa-50 kDa-20 kDa) trypsin split myosin subfragment-1, which could no longer be activated by actin, did not bind to the two sites located in the 96–125 region, but it still interacted with the 338–348 and 360–372 segments [19].

The presence of hydrophobic interactions in the actin–myosin complex have already been suggested [21,22].

A multisite interface model has been suggested [17]. This model obviously did not take into account the location and simultaneous accessibility of myosin-binding sites on adjacent actin monomers nor the two real discontinuous ATP-dependent binding sites on the interface [19] and hydrophobic cluster organization. The present study was focused on this topic.

2. Materials and methods

Two-dimensional hydrophobic clusters analysis (HCA), recently developed by Mornon's French team (University of Paris 6) is based on a representation of protein sequences on an α-helical 2D pattern, using secondary structural analysis, comparing the distribution of hydrophobic clusters along the sequence. The first examination of the HCA plot does not involve reading the sequence itself but rather identifying the structural segmentation constituting the hydrophobic core of globular protein domains. Plots are thus examined to analyse the horizontal distribution and size of the various hydrophobic clusters. HCA uses a highly degenerated code for the sequence with only two main states initially considered: hydrophobic and hydrophilic. No definite window was used. Highly hydrophobic (VILF) or moderately hydrophobic (WMY) residues tend to form clusters of various shapes and sizes [23]. Clusters of similar shapes, sizes and relative positions express similar patterns in polypeptide folding of the protein.

HCA offers the following advantages: (a) the 2D plots highlight remote information visible more readily than with methods based only on single amino-acid property/identity; (b) deletions or insertions are easily introduced in the secondary structure. HCA is an effective method for analysing proteins, as recently illustrated for a number of proteins [23–25].

In our case, the HCA method could be an efficient tool for analysis of the actin molecule since the crystallographic actin model is available. Knowledge on the 3-D structure (Fig. 1) and mutations are therefore the most useful way for deriving structural and functional information through homology HCA modelling.

The following actin sequences were extracted using the Bisance program [26] for access to the Protein Information Resource (NBRF-PIR): skeletal muscle (rabbit), soybean, sea urchin (*Strongylocentrotus purpuratus*), cytosolic-starfish (*Pisaster ochraceus*), cytosolic A3-silkworm

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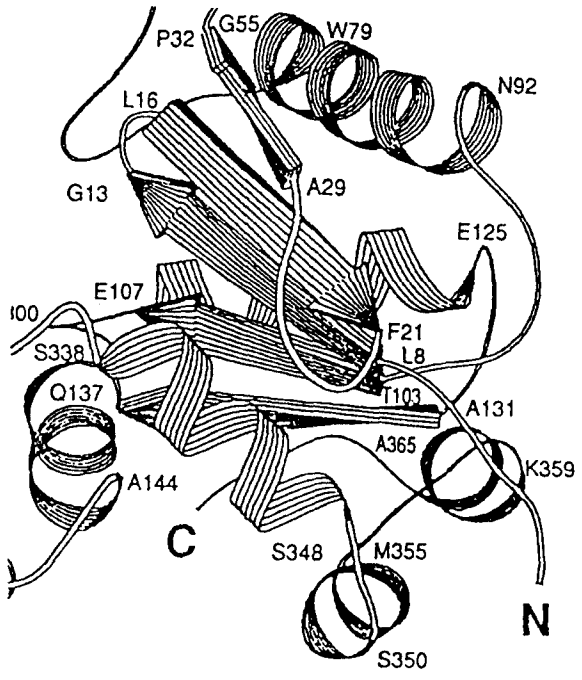

Fig. 1. Rabbit skeletal actin according to Kabsch et al. [2].

(*Bombyx mori*), slime mold (*Physarum polycephalum* and *Dictyostelium discoideum*), *Caenorhabdtis elegans*, *Tetrahymena pyriformis*, imperfect fungus (*Candida albicans*), *Acanthamoeba castellanii*, smooth muscle (human, rat, chicken), β-actin (chicken, human, mouse, rabbit), γ-actin (chicken, human, mouse, enteric human), cardiac muscle (chicken), fruit fly (actin 7, actin 8, actin 87E) (*Drosophila melanogaster*), *Hydra attenuata*., macronuclear (*Oxytrica nova*), *Entamoeba histolytica*, B-actin (human).

3. Results

3.1. Extensive HCA of rabbit skeletal actin

We analysed and compared 27 actin sequences by HCA method and deduced HCA patterns. These sequences are representative of the phylogenetic tree of actins [27].

The upper part of Fig. 2 illustrates the distribution of hydrophobic clusters along the amino-acid sequence of actin sequence. The lower part of Fig. 2 illustrates the primary and secondary structures corresponding to clusters. The HCA plot patterns of rabbit skeletal muscle actin revealed two groups of identical hydrophobic motifs.

The first one includes a motif (A_1) located in the region between residues 70 and 90, except for *Tetrahymena* actin, and an identical motif (A_2) located in the 320–375 fragment in the region between residues 335 and 358, except for *Oxytrica nova* actin. The second group includes a motif (a_1) located in the region comprised between residues 90 and 115 and an identical one (a_2) between residues 358 and 375.

We observed that amino-acid variations in actin isoforms did not alter the HCA pattern of these conserved motifs (A, a) for any of the actins studied, except for actin from *Macronuclear oxytricha*. This distribution of identical hydrophobic clusters on the same polypeptidic chain was not observed in many other globular domains.

3.2. Precise local analysis of hydrophobic motifs A and B in actins

The hydrophobic cluster pattern observed by HCA showed the presence of two large motifs.

The motif A_1 was constituted of contiguous residues (I75, I76, W79, M82 and I85) and motif A_2 (I341, I345, L349 and F352) of similar contiguous residues. Hydrophobic residues of motif A_2 were included in the interface of one actin monomer with S1 and motif A_1 was described as being included in the interface of S1 with a second monomer as seen in 3D atomic model of actin-S1 [4].

Indeed, in the atomic structure of actin [2], one α-helix occurs between residues 79 and 92 and another one between residues 338 and 348. These two α-helices are outside of subdomain-1 [2] and bear two myosin-binding sites that are effective in the presence of ATP-Mg [5,28], contrary to binding sites on the loops (sequences 21–29, 96–103 and 360–369) which are ineffi-

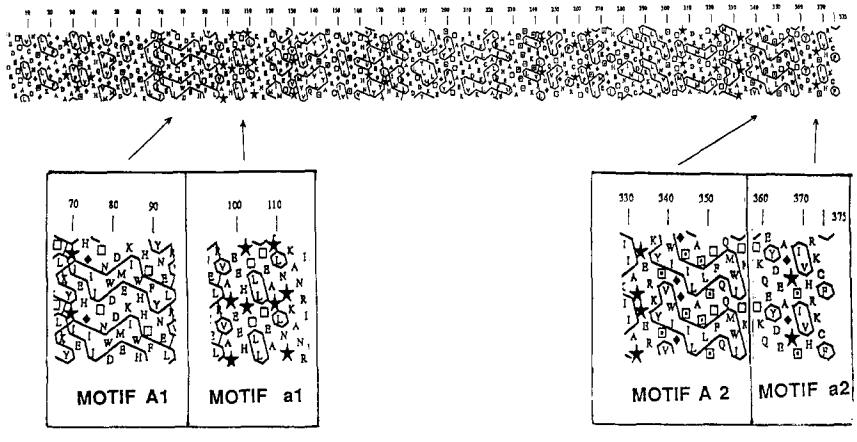

Fig. 2. Upper part: HCA plot of actins. Numbers and vertical lines correspond to amino-acid positions. (a) *Tetrahymena pyriformis*, (b) yeast, (c) *Dictyostelium dicoideum*, (d) rabbit. Lower part: enlarged presentation of the HCA plot of the conserved motifs in the actin monomer (vertical marks). The first motif (A_1 and A_2): A_1 was constituted by hydrophobic residues (shaded area) of the α-helix (79–92): A_2 was constituted of hydrophobic contiguous residues (shaded area) L349, F352 and I341, I345 of the α-helix segment (residues S338 to S348).
The second motif (a_1 and a_2); a_1 was constituted by sequence residues N92 to P112 comprising hydrophobic residues V96, L110 and clusters L104 and L105. a_2 constituted by sequence K359–F375 comprises hydrophobic residues Y362, F375 and the cluster (I369, V370). Identification of amino acids: ☆, P; ◆, G; □, T; ⊡, S.

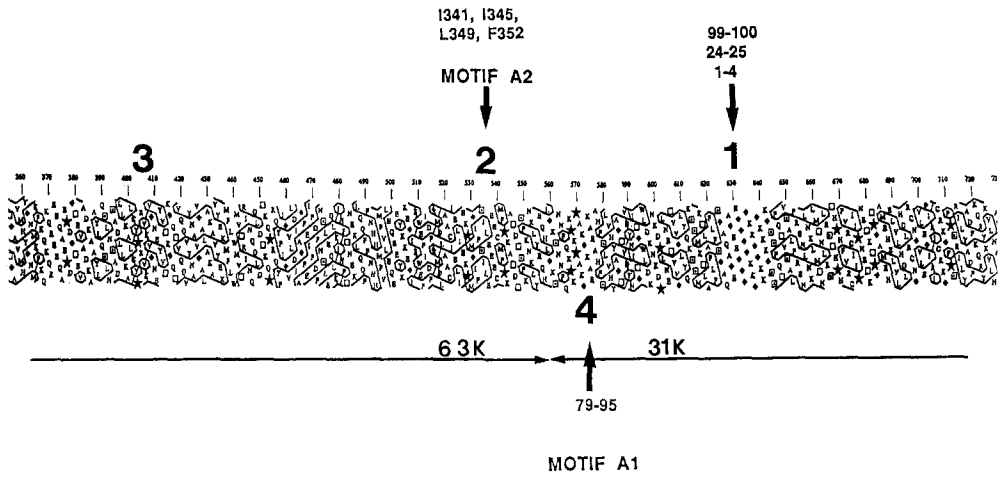

Fig. 3. HCA plot of skeletal rabbit myosin S1. Number and vertical lines correspond to amino-acid positions. The sequences corresponding to the three actin contacts [4] are specified by 1, 2, 3, with a first actin monomer and the fourth contact with a second actin monomer.
Contact 1: residues 626 to 647 S1 of the myosin sequence are in contact with actin residues D1, E2, E3, D4, D24, E25, D99 and D100.
Contact 2: residues P529, I530, I535, M541, F542, P543 of myosin sequence are in contact with exposed hydrophobic residues I341, I345, L349 and F352 (motif A2).
Contact 3: loop formed by residues 403–416 of the myosin sequence in contact with actin residues P332 and P333.
Contact 4: loop formed by residues N567–H578 of myosin sequence in contact with the C-terminus of α-helix formed by residues W79–R95 of actin (motif A1).

cient in the presence of nucleotides [19]. Natural mutations at residues 89 and 358 occur in smooth muscle actin and cause variations of the K_m value in the actin-activated Mg-ATPase of skeletal myosin [29].

Myosin subfragment-1 could present motifs as a counterpart in the interaction with two different monomers in the absence or presence of nucleotides. HCA pattern, which is similar for scallop and rabbit myosin-S1, showed the four major contacts with actin monomers (Fig. 3).

The HCA plot of scallop or rabbit myosin S1 sequences (560–580) revealed the presence of the particulary loop at the junction N-terminal 63 kDa and the C-terminal 31 kDa [4]. Actin residues 144, 341, 345, 349 and 352 (motif A_2) located on one monomer were shown by EM and image analysis [3] to be in contact with residues 529, 530, 535, 541 and 542 (contact 2) located in the N-terminal 63-kDa fragment of S1 (Fig. 3) and the actin 79–92 sequence on the other monomer (motif A_1) was found to be in contact with the myosin 567–578 (contact 4) sequence in the N-terminal part of the 31-kDa segment (Fig. 3). This suggested that there could be a major contact with S1 in the presence or absence of ATP taking place on each monomer, on segments 338–348 and 79–92, respectively. Motif A_1 and motif A_2 take place on each side of the 63-kDa–31-kDa junction.

3.3. *Precise local analysis of hydrophobic motifs a and b*

The HCA pattern showed the presence of similar motifs a_1 and a_2. The first one was constituted of residues (V96, L104, L105 and L110) in the 90–110 fragment and motif a_2 was constituted of residues (Y362, I369, V370 and F375) included in the 360–375 fragment. These hydrophobic residues were strictly conserved during the evolution of actin and we consider that they could be implicated in the rigor complex.

Natural actin isoforms or induced mutants were previously studied in connection with acto–myosin interactions. Concerning actins from invertebrates (*Pecten maximus*), amino-acid substitutions at residues 103, 358 and 365 [30] are located in motifs a_1 and a_2 and two of them (103 and 365) concern ATP-dependent binding sites. We demonstrated [31] that these mutations were responsible for a decrease in the V_{max} of actin-activated Mg-ATPase of skeletal myosin. A few induced mutations in *Drosophila* were obtained (G368E and E316K), resulting in normal muscle structure while reducing S1 rigor binding [32], and altering the kinetics of force generation [33]. In contrast, substitution by histidine at E360/E361 and D363/D364 in *Dictyostelium* actin [34] (motif a_2) resulted in wild-type motility and affected the kinetic steps of the ATPase cycle. When E93K mutation was induced, actin bound the myosin head in the rigor complex [35]. Conversely, when amino residues E99/E100 (motif a_1) in *Dictyostelium* actin were replaced with histidine residues, the velocity of filament sliding in vitro was only 17% of that of wild-type F-actin [34].

4. Discussion

2D HCA of 27 actin sequences suggested the presence of two very similar hydrophobic clusters (motifs A_1 and A_2) in the monomer including previously observed ATP-independent myosin-binding sites (70–90 and 338–358 fragments).

We showed [36] that splitting of scallop myosin S1 heavy chain occurred between the 63-k N-terminal and the 31-k C-terminal segments and was associated with acto-Mg-ATPase inhibition (contact 4). Such a split heavy chain could be obtained with chemically modified skeletal rabbit S1 [37]. This connection is important in communications between actin- and nucleotide-binding sites and the structural freedom of this region may be critical for conformational transition during ATP hydrolysis and actin interaction [38]. In contrast to filamentous actin, the monomer is unable to hinder limited trypsin proteolysis of S1 [39].

In our model, the two similar hydrophobic clusters (motif A_1 situated on actin a_2 and motif A_2 situated on actin a_1) were in contact (contacts 4 and 2, respectively) on each side of the myosin subfragment-1 63 kDa–31 kDa connecting segment (Fig. 3).

A conformational change in the 96–103 segment was induced on the actin monomer by the binding with anti-(18–28) antibodies [17], thus increasing exposure of residues in this segment (contact 1). This indicated a dynamic relationship essential in the motility process [40]. This negative cluster and the proximal hydrophobic cluster, including residues of the 338–348 helix, are associated in the first monomer. On the adjacent monomer, the second hydrophilic cluster (helix 79–92) and the two other ATP-dependent sites (112–125 and 360–369), which project on the same side of subdomain-1, could be included in the same interacting region. The assumed presence of a ternary complex ($actin_2$-$S1_1$) proposed [8,9] suggests that the environment of Cys374 on one of the two actin molecules is more affected than the other after S1 binding. Since Cys374 occurs close to helix 338–348 [28], there could be an asymmetrical interaction of S1 with each monomer. Such interactions involving two anchorage regions must impose a specific topology through a conformational constraint on F-actin by S1, and the presence of ATP might decrease or hinder the interaction with one of the two monomers during the gliding process. Internal cross-linking of a monomer inhibits movement but not myosin binding or Mg-ATPase activation [41] probably by chemically freezing the flexibility of the filament in the presence of myosin [42]. We postulate that some relation and message between the two motifs A_1 and A_2 seem probable through the S1 (63 kDa–31 kDa) connecting segment. This proposal is currently investigated.

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