DNAse I - actin complex: An immunological study

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DNAse I - actin complex formation is studied in the presence of different anti actin antibody populations. The binding of DNAse I to actin is shown to be affected by antibodies specific to a central region in actin sequence (168-226). The C- and N-extremities of actin are shown to be in spatial proximity at the surface of the actin monomer and far from the binding area of DNAse I.

Actin is a major constituent of muscle and non-muscle cells. It appears as a multifunctional protein which occurs not only as microfilaments crosslinked by high-molecular-weight proteins (1), but also as monomeric species stabilized by low-molecular-weight proteins such as profilin (2-4) and DNAse I (5).

In vitro DNAse I is known (6-7) to interact strongly with G-actin in a 1:1 complex leading to the inhibition of DNAse I enzymeric activity and preventing actin polymerization. Crystallographic studies (8) at low resolution of this complex evidenced a large contact area between the two proteins and showed the occurrence of two lobes within the actin molecule.

A previous study (9) showed that a large N-terminal fragment (mol.wt. about 25 000) isolated from actin digest was able to interact with DNAse I. Moreover, Suto (10), using a crosslinking reagent, localized one of the contact points in the segment 48-92.

The aim of this study is to examine the actin sequences concerned with DNAse I contact, using specific anti-actin antibodies directed against different parts of actin. In particular, the N- and C-extremities of the polypeptide chain appear topographically independent from the DNAse I binding site.

Materials and Methods

Rabbit skeletal muscle and scallop adductor muscle actins were prepared according to the method in reference 11. Protein concentrations were obtained spectrophotometrically (12,13).
Actin was labelled at cysteine 374 (14) by N-iodoacetyl-N-(5-sulfo-l-naphthyl)ethylenediamine (1,5-I-AEDANS) and cleaved at cysteine residues by 2-nitro-5-thiocyanobenzoic acid (15). Actin was proteolyzed by Staphylococcus aureus V8 protein (16-18) and trypsin (19).

The small N-terminal tryptic peptides (related to the 1-67 residues of actin) were separated from actin core (19) by acidic precipitation. Large actin fragments were purified by electrophoresis on SDS slab gels (20) on an Isco sample concentrator, model 1750. N-terminal tryptic peptides, C-terminal 16 000 fragment, G-actin, carboxymethylated unfolded actin and dansylated bovine serum albumin were coupled to Sepharose 4B using the CNBr procedure (21). Antibodies induced by performic-acid-oxidized (OxA) or trinitrophenylated (ArA) unfolded actins were obtained as previously described (17). Antibodies directed towards C-terminal 10 000 fragment derived from selective cleavage of actin at Cys 285 were elicited in sheep. Before immunization, this purified fragment was coupled to hemocyanin (22). Anti AEDANS antibodies were elicited in sheep using dansylated arginine kinase (23,24) as immunogen and purified on insolubilized dansylated serum albumin.

Peptide transfer on nitrocellulose sheet was performed as in reference 17. A radioimmunoassay (25) was used to follow the interaction between actin monomers and sheep antibodies. Briefly, insolubilized G-actin was incubated for 1 h with increased amounts of sheep antibodies. After three washes in 0.5-M NaCl, 0.05% Tween 20, 10 μl of rabbit antiserum G (IgG) serum (Nordic) were added. Then, in a third step, 50 μl of 125I-labelled Protein A (about 40 000 c.p.m.) were added as tracer. The amount of radioactivity bound to the washed solid phase is a measure of antibody binding to actin monomers.

DNAse I activity in the supernatant was measured spectrophotometrically according to the method in reference 26, at 25°C in 5 mM MgSO4, 0.1 M sodium acetate buffer pH 5.0, using 4 μg ml⁻¹ of DNAse I and 100 μg ml⁻¹ of DNA.

Results

The specificity of the various lots of antibodies makes it possible to distinguish between three domains in the primary structure of actin.

N- and C-terminal regions

The N-terminal end of actin can be mapped by anti ArA antibodies; the small insolubilized N-terminal tryptic peptides (related to the 1-67 sequence) were used to purify an N-terminal-specific population. We observed that about 70% of these antibodies were retained on the immunoabsorbant and are therefore directed towards this actin sequence (Fig. 1). The specificity of this purified population is confirmed in the experiments presented in Fig. 2A. These antibodies do not recognize the actin core lacking the 62 first amino acids. Furthermore actin from scallop which strongly differs from skeletal actin in sequence 1 to 10 (27) is not recognized by these antibodies (Fig. 2A). These results indicate that sequence 1-10 constitutes the antigenic region related to this population.
Fig. 1. Ordering of the different fragments in the actin primary structure (black bar) and location of the antigenic regions (xxx).

Fig. 2. Antigenic reactivity of electrophoretic replicas from actin or actin digest. (A) Reaction of the N-terminal antibody population derived from anti ArA antibodies with (a) actin from rabbit muscle, (b) tryptic digest of actin from rabbit muscle, (c) actin from scallop muscle. (B) Reaction of anti OxA antibodies with S. aureus V8 protease digest of actin from rabbit muscle. 1, SDS-15% (A) or 12% (B) polyacrylamide slab gels (Coomassie blue staining); 2, electrophoretic replicas.
The C-terminal domain of actin was studied using antiserum elicited by a C-terminal fragment of actin. This fragment was obtained after chemical cleavage of actin labelled at Cys 374 by the fluorogenic reagent 1,5-I-AEDANS. Analysis on SDS slab gel of the hydrolysate shows at least 7 released fragments whose apparent molecular weight lies between 33 000 and 10 000 (Fig. 3). Residual actin and three fragments appear fluorescent. They would be attributed to the C-terminal end of actin and constitute overlapping fragments. The smaller fluorescent peptide, from its molecular weight (10 000) and the specificity of the cleavage appears to correspond to the sequence 285-375 (Fig. 1). The antibodies elicited against this fragment were purified on immobilized G-actin. On the other hand, an additional antigenic site was artificially created in the C-terminal extremity by coupling the AEDANS haptenic group at Cys 374 (Fig. 1). Then the purified anti dansyl antibodies can specifically react at the C end of actin.

The effects of these antibody populations were tested towards DNAse I - actin interaction. As shown in Fig. 4, DNAse I can be almost completely inactivated after incubation with the immobilized G-actin. A similar result is obtained with dansylated G-actin. An apparent $K_D$ in the range of $10^{-7}$ M can be estimated for DNAse I binding in our experimental conditions. The interaction of DNAse I with G-actin was affected (Fig. 4) neither by the N-terminal (sequence 1-10) specific population nor by the C-terminal specific populations.

![Fig. 3. Chemical cleavage by 2-nitro-5-thiocyanobenzoic acid of 1,5-I-AEDANS labelled actin. SDS-15% polyacrylamide slab gel. Mol. wt. standards: thrombic digest of actin (32). (a) Total hydrolysate, (b) detection of fluorescent fragments, (c) purified peptide (10 000 mol. wt.) used for immunization.](image-url)
Fig. 4. Effect of specific antibodies on the binding of DNAse I to insolubilized actin. Sepharose 4B immobilized G-actin or AEDANS-G-actin (100 μg) was incubated in 20 mM Tris, 10 μM bovine serum albumin buffer pH 7.5 for 30 min with specific antibodies, then for 5 min, with DNAse I (50 μg) and centrifuged. DNAse I activity (see Materials and Methods) is monitored at 260 nm in the supernatant. Increase in absorbance is followed versus time. References: experiments without antibodies (○) or with Sepharose 4B alone (★). Samples: reaction with N-terminal specific antibodies (1 mg) (▲), anti dansyl antibodies (1 mg) (△), anti 10000 C-terminal fragment antibodies (1 mg) (■), anti OxA antibodies (1 mg) (●) and anti 16000 C-terminal fragment antibodies (0.2 mg) (□).

Thus, further attempts were made to show the accessibility of the two extremities of actin molecule and their location towards DNAse I binding area. Fig. 5 shows that the two extremities near Cys 190 and Cys 374 are recognized in G-actin by the related specific antibodies. In addition, these antibodies are not able to bind together on the G-actin.

Central part of actin

The third domain in actin, the central part, is mapped by anti OxA antibodies. Their specificity was definite towards S. aureus V₈ protease digest of actin (17,18). Fig. 2B summarized the results described previously (18). It shows that anti OxA antibodies react with the two major fragments but not with the 19 000 mol.wt. N-terminal subfragment resulting from a secondary cleavage site at Glu 167. Thus these antibodies are specific to the sequence 168-226 and 227-375. Furthermore, as only 20% of them are retained on the 16 000 C-terminal fragment linked to Sepharose 4B, these anti OxA
antibodies are essentially specific to the central part of actin. In contrast with preceding antibodies, they affect DNAse I binding (Fig. 4). About 50% protection is observed with an antibody/actin ratio (mol/mol) of 2. The antibodies specific to the sequence 227-375 were purified on the insolubilized C-terminal 16 000. Used at the concentration occurring in the initial antibody population (Fig. 4), they are not effective. Therefore, this population does not account for the effect afforded by anti OxA antibodies.

**Discussion**

The interaction of DNAse I with actin is known to be highly specific. The affinity of pancreatic DNAse I for monomeric actin has been reported and lies between $2 \times 10^{-9}$ and $6 \times 10^{-7}$ M (28,29). We have noticed that DNAse I and monomeric actin linked to Sepharose 4B still interact with a high affinity. In agreement with other reports (29,30), this interaction does not seem significantly altered by Cys 374 modification. Thus insolubilized actin appears a suitable tool to afford further information about the spatial structure of actin in connection with the actin - DNAse I interaction area.

From our results it appears that the N extremity of actin near Cys 10 and the C extremity including Cys 374 are in spatial proximity as judged by the competition observed between antibodies directed towards sequence 1-10 and the dansylated cysteine. We demonstrate also that these two terminal ends of actin are exposed on the surface of G-actin and are not located within the large DNAse I - actin
contact area. These results extend the previous observations of Suck et al. (8) which locate Cys 10 in the smaller lobe of actin (31) and underline the tryptic susceptibility of Lys 373 in DNAse I - actin complex. Moreover, we show that the interaction between actin and antibodies directed towards a part of the sequence 168-226 affects the binding of DNAse I. This result suggests that an antigenic determinant of actin carried by sequence 168-226 is included in (or near) the contact area between actin and DNAse I. It should be noted that two previous studies show that first, the reactivity of this antigenic determinant is affected by the structural transition induced by Mg2+ (18) which promotes the polymerization process, and second, that its interaction with anti OxA antibodies drastically decreases the polymerization rate of actin (17). From the present work, this antigenic determinant also appears to be related to the contact area between actin and DNAse I.

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