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 enzymic 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 from 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 antishell immunoglobulin 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.