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Competitive binding of the troponin T-specific pool of caldesmon antibodies and tropomyosin to skeletal troponin T and smooth muscle caldesmon

Konstantin G. Birukov, Vladimir P. Shirinsky, Alexander V. Vorotnikov* and Nikolai B. Gusev*

Institute of Experimental Cardiology, USSR Cardiology Research Center, USSR Academy of Medical Sciences, Moscow 121552 and **Department of Biochemistry, School of Biology, Moscow State University, Moscow 119899, USSR*

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The fraction of polyclonal caldesmon antibodies cross-reacting with rabbit skeletal troponin T are shown to compete with smooth muscle tropomyosin for caldesmon and troponin T, as revealed by ELISA method. The epitope recognized by these antibodies was also found in M_r 77 kDa non-muscle caldesmon. These results provide functional confirmation for the suggestion that the regions of amino acid sequence homology in caldesmon isoforms and troponin T belong to the tropomyosin binding sites.

Caldesmon; Troponin T; Tropomyosin

1. INTRODUCTION

Caldesmon and troponin are structurally different but functionally similar proteins involved in the thin filament-based system for the regulation of contraction in smooth and striated muscles, respectively [1-3]. It has been recently shown that caldesmon and the troponin T subunit of skeletal troponin share common antigenic determinants and the sites with high degree of primary structure homology [4-6]. These amino acid sequences were suggested to belong to tropomyosin binding site. In the present work we confirm this suggestion by demonstrating that tropomyosin competes with the troponin T-specific pool of caldesmon antibodies for common sites on skeletal troponin T and smooth muscle caldesmon. We have demonstrated also that similar epitopes are present in non-muscle caldesmon.

2. MATERIALS AND METHODS

All reagents used were of analytical grade. The following proteins were purified by previously described methods: chicken gizzard caldesmon [7], rabbit liver caldesmon [8], rabbit skeletal troponin T [9] and chicken gizzard tropomyosin [10]. Chymotrypsinolysis of caldesmon by α-chymotrypsin was performed according to Szpacenko and Dabrowska [11]. Protein coupling to CNBr-activated Sepharose-4B (Pharmacia) was performed according to the manufacturer's instructions. Polyclonal antibodies to chicken gizzard smooth muscle caldesmon were characterized earlier [4]. The troponin T-specific pool of caldesmon antibodies (TNT-S AB) was affinity-purified on troponin T-Sepharose 4B. By ELISA, TNT-S AB demonstrated no cross-reaction with immobilized tropomyosin. SDS-PAGE was performed according to the method of Laemmli [12] and electroblotting procedure was accomplished as described by Towbin et al. [13].

Competitive binding was assessed by ELISA method. Smooth muscle caldesmon (0.5 μg/ml) and skeletal muscle troponin T (4.0 μg/ml) were immobilized in 96-well microtitration plates by incubation for 3 h at 37°C in 0.01 M carbonate-bicarbonate buffer (pH 9.6) in a humidified atmosphere. Unbound material was washed out with phosphate buffered saline (PBS) containing 0.05% Tween-20. Tropomyosin (10 μg/ml) in PBS/Tween was incubated for 30 min at 37°C followed by the addition of TNT-S AB in PBS/Tween. Alternatively, the mixture of 8 μg/ml TNT-S AB with various concentrations of tropomyosin was added to the wells and incubated for 30 min at 37°C. Unbound proteins were washed out with PBS/Tween. To reveal TNT-S AB binding, second antibodies conjugated with horseradish peroxidase (10 μg/ml) were added to the wells and incubated at room temperature for 1 h. Orthophenyldiamine (2 mg/ml) was used as a substrate for peroxidase reaction. Optical density was read at 492 nm using a Titertek Multiskan automatic microphotometer (Flow, U.K.).

3. RESULTS AND DISCUSSION

Figs. 1, 2A show that interaction of tropomyosin with immobilized caldesmon and troponin T attenuates the binding of TNT-S AB to these proteins. As shown in figs.1, 2B, tropomyosin added together with TNT-S AB inhibits the binding of antibodies to caldesmon and troponin T in a concentration-dependent manner. Maximum inhibition of the TNT-S AB binding to caldesmon and troponin T was reached at 40-fold and 5-fold molar excess of tropomyosin over the antibodies, respectively.

Purified M_r 77 kDa caldesmon from rabbit liver was positively stained by troponin T-specific antibodies in immunoblotting (inset in fig.1B).

Correspondence address: K.G. Birukov, Institute of Experimental Cardiology, Cardiology Research Center of the USSR, 3rd Cherepkovskaya Street 15 A, Moscow 121552, USSR

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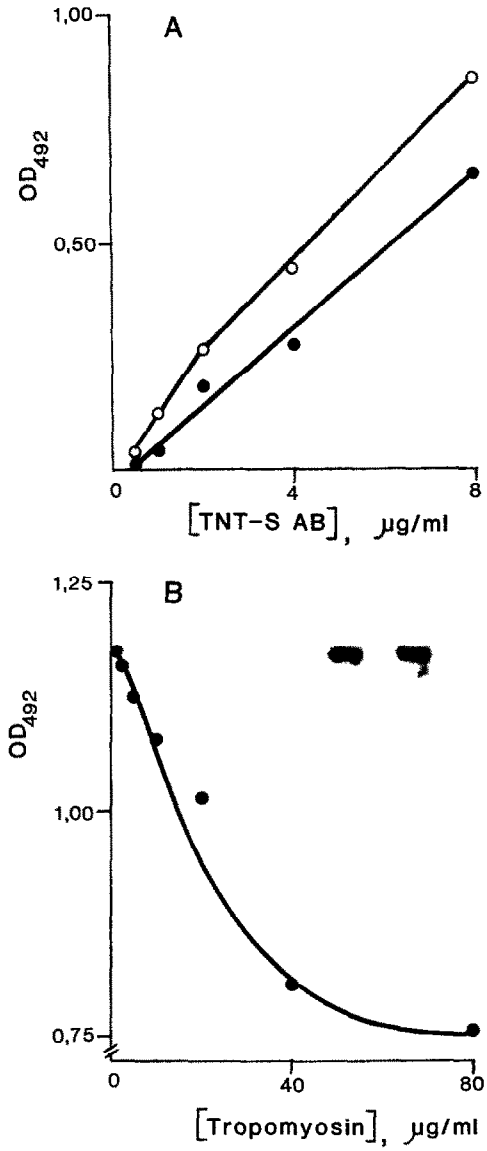

Fig.1. The effect of tropomyosin on the binding of TNT-S AB to immobilized caldesmon. (A) Titration of immobilized caldesmon (○) and caldesmon preincubated with tropomyosin (●) by TNT-S AB. (B) Titration of immobilized caldesmon by tropomyosin in the presence of 2 µg/ml TNT-S AB. Inset shows SDS PAGE track of M_r 77 kDa caldesmon (left) and corresponding blot, stained with TNT-S AB (right).

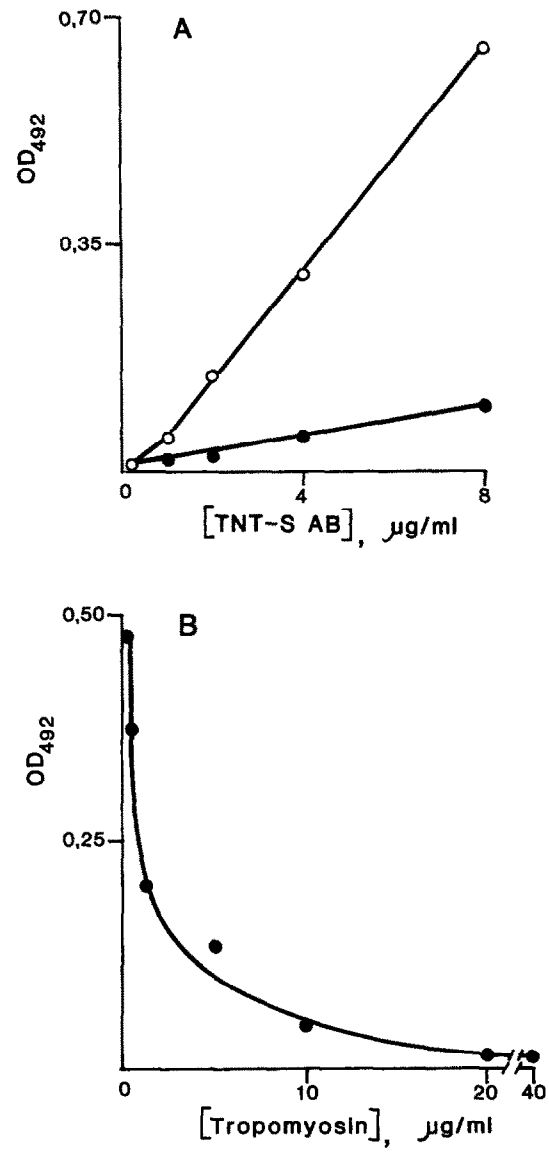

Fig.2. The effect of tropomyosin on the binding of TNT-S AB to immobilized troponin T. (A) Titration of immobilized troponin T (○) and troponin T preincubated with tropomyosin (●) by TNT-S AB. (B) Titration of immobilized troponin T by tropomyosin in the presence of 8 µg/ml TNT-S AB.

The inhibition of TNT-S AB binding to immobilized caldesmon or troponin T by tropomyosin may be explained by competition of antibodies and tropomyosin for common binding sites on caldesmon and troponin T. With immobilized caldesmon, the antibody binding to it could not be prevented completely even at high concentrations of tropomyosin (fig.1B). Presumably, there is a pool of high affinity binding antibodies in TNT-S AB preparation which cannot be displaced by tropomyosin at concentrations used, since the affinity of tropomyosin to caldesmon is weak (approx. K_a 10^5 M^{-1}) [14].

In contrast, when caldesmon was substituted by troponin T, the entire inhibition of troponin T - TNT-S AB binding was achieved at 5-fold molar excess of tropomyosin over antibodies. These findings suggest that the affinities of TNT-S AB and tropomyosin to the binding sites on immobilized troponin T are comparable to each other. Caldesmon may also contain several tropomyosin binding sites with different affinities to tropomyosin and TNT-S AB. We observed that TNT-S AB stained only the portion of M_r 150 kDa caldesmon chymotryptic fragments revealed by original caldesmon antibodies. Among those fragments M_r 38 kDa C-terminal caldesmon fragment reacted positively with TNT-S AB (data not shown). These findings are

consistent with the mapping of tropomyosin binding site in the C-terminal fragment of smooth muscle caldesmon on the basis of the amino acid sequence analysis [15]. TNT-S AB reacted with M_r 77 kDa caldesmon in immunoblotting (inset in fig.1B). Thus, M_r 77 kDa caldesmon also contains amino acid sequences similar to those found in smooth muscle caldesmon and troponin T and involved in tropomyosin binding.

In conclusion, common amino acid sequences revealed in caldesmon and skeletal troponin T were suggested to be involved in tropomyosin binding. The present work confirms the primary structure data by functional tests on the binding of tropomyosin to the similar sites on caldesmon and troponin T.

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