Effect of the detergent C₁₂E₈ on the binding of monoclonal antibodies to the (Ca²⁺·Mg²⁺)-ATPase of rabbit skeletal sarcoplasmic reticulum

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Introduction

It is generally accepted that although the (Ca²⁺·Mg²⁺)-ATPase can function as a monomer, it exists in an oligomeric form (probably a dimer) in vivo [1]. The nature and location of the protein–protein interactions involved in oligomerization are unclear, but one way in which such interactions may be investigated is to use monoclonal antibodies (mAbs) to interfere with these interactions. In this study, we present evidence which suggests that the epitopes binding to the nucleotide-binding domain of the ATPase are located close to the sites of interaction between ATPase dimers.

Results and discussion

A library of mAbs has been produced against the (Ca²⁺·Mg²⁺)-ATPase, of which several are inhibitory. According to competition-binding studies these inhibitory mAbs appear to bind to a similar region of the protein [2].

Figure 1 shows the effect of these mAbs on the activity of the membraneous (Ca²⁺·Mg²⁺)-ATPase. The maximal observed level of inhibition of ATPase activity is between 30 and 50% with the exception of 1/2H7 which had no effect on activity and is included as a control. In the presence of 2.5 mg of C₁₂E₈/ml, which monomerizes the ATPase, the inhibition of this effect of these antibodies was significantly increased, consistent with increased levels of binding of the antibodies to the ATPase and thus increased levels of inhibition. The increased inhibition cannot be attributed to denaturation of the ATPase in the presence of C₁₂E₈ (which is indeed seen at long incubation times), since no significant inhibition of ATPase activity was observed for mAb 1/2H7 in the presence of C₁₂E₈. Enzyme-linked immunosorbent assay (ELISA) studies (not shown), in which ELISA plates were coated with both native and SDS-denatured ATPase, showed that more sites were available for binding these inhibitory antibodies in the denatured as compared with the native form of the protein, suggesting that in the native ATPase the epitopes of these inhibitory antibodies were masked.

All of these mAbs have been mapped by Western blotting of peptide fragments to that part of the B fragment of the ATPase which is thought to constitute the nucleotide-binding domain [3]. Some of these antibodies have recently been mapped more accurately using synthetic peptides (R. E. A. Tunwell, J. M. East & A. G. Lee, unpublished work) confirming our original assignment of epitopes.

These data indicate that the inhibitory antibodies bind to a site which is unmasked by the monomerizing effect of C₁₂E₈. We suggest that in the dimeric form of the ATPase (which contains two epitopes), the two epitopes are located close together. This would be close to the site of protein–protein contact between the ATPase molecules. The binding of one mAb would then sterically inhibit the binding of a second antibody to the same dimer. In the presence of C₁₂E₈, monomerization of the ATPase molecules would then allow each ATPase molecule to bind an antibody.

In the case of B/3D6, we suggest that the binding of one mAb per ATPase dimer precludes the binding of further mAbs resulting in a doubling of the inhibition seen when the ATPase is monomerized by C₁₂E₈. The effect of C₁₂E₈ is less marked for the other antibodies, but still suggests that steric hindrance probably prevents binding to all of the available epitopes in the dimeric form of the ATPase.

Since these mAbs bind to the nucleotide-binding domain, these results suggest that the nucleotide-binding domains could be in close proximity to each other in dimeric ATPase and may constitute the point of contact between dimers. Electron micrographs of two-dimensional crystals of the ATPase show that the point of contact between dimers is approximately 42 Å above the plane of the lipid bilayer [4] and it has also been shown by energy-transfer studies that the nucleotide-binding site is located 80 Å above the plane of the bilayer [5], so that contact between the nucleotide-binding domains would be possible.

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Abbreviation used: mAb, monoclonal antibody.