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Development of Immunological method for Monitoring physico-chemical changes of Muscle Myosin. I. Establishment and preliminary application of Ci-ELISA to myosin subfragments

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ABSTRCTS

In ordor to understand physico-chemical properties of myosin molecules, we have produced antisera to myosin heavychain whole molecules (200 kDa, as monomer) and to myosin subfragments which were prepared by α-chymotrypsin treatment. There are three myosin subfragments ; heavymero myosin S-1 (myosin head part, 96 kDa), heavymero myosin S-2 (48 kDa) and lightmero myosin (56 kDa). We immunized rabbits with S-1 and S-3. Boosting injection was done after 4th injection of antigen of antigen (0.1㎎/㎖). Antisera from rabbit were collected and IgGs were purified by affinity chromatography using Protein A sepharose column. To format the condition of Competitive indirect Enzyme Linked Immunosorbent Assay (Ci-ELISA), plates were coated by myosin and blocked by 1% BSA (bovine serum albumin). The following condition appeared to bring the good results ; anti-S-1 and anti-S-3 were diluted 1:1000, anti-myosin was diluted 1:2000. HRP (Horseradish peroxidase) conjugated Goat anti rabbit IgG were diluted 1:10,000 ($p<0.05$). The ranges of precisional application on the standard of S-1, S-3 and myosin were from 4.0 ㎍/㎖ to 250 ㎍/㎖ and the detection limits at O.D 490nm was 0.1 ㎍/㎖($p<0.05$). The cross reactivity between antigen S-1 and anti-S-3 IgG was 10.3 % and the cross reactivity between antigen S-3 and anti-S-1 was 8.6 % ($p<0.05$). Recovery tests of Ci-ELISA were myosin : 101.7%, S-1 : 102.6%, S-3 : 101.6% ($p<0.05$). With this assay method in our hand, we monitored the rate of thermal denaturation of bovine myosin. As the temperature of myosin solution (125 ㎍/㎖) increased to 90℃, the rate of reaction of each IgG to myosin molecule markedly decreased. The epitopes of myosin whole molecule were conserved 59.29% and 12.27% at 60℃ and 90℃ respectively compared to normal 37℃. The reactivities of anti-S-1 antibody were abruptly decreased at 50℃, and those of anti-S-3 antibody at between 40℃ and 60℃ ($p<0.05$). The amount of Ag detected from anti-S-1, anti-S-3 and anti-myosin IgG were different in thermal denaturation. This study presents that Ci-ELISA can be used not only at diagnostic fields but also at the reserch of certain molecules characterization.

Key words : myosin, myosin subfragments, Ci-ELISA, thermal denaturation