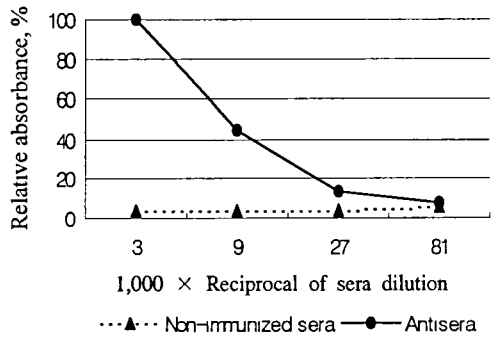
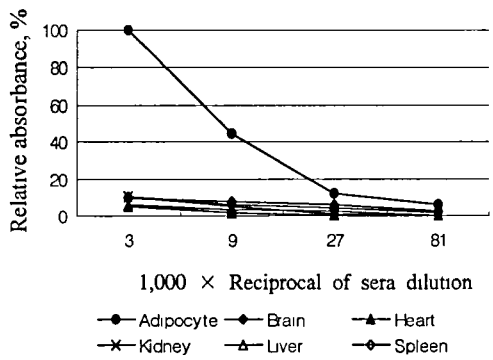
specificity. Plasma membrane proteins from adipocyte, brain, heart, kidney, liver, and spleen were isolated using a self-forming Percoll gradient. Sheep (40kg) was immunized three times at three week interval with the purified APM proteins. Blood was taken from non-immunized sheep (NS) and from immunized sheep at 10 (AS-1), 12 (AS-2), and 14(AS-3) days after the third immunization. Antisera titers and cross-reactivity against other tissues were determined by enzyme-linked immunosorbent assay (ELISA). Antisera reacted strongly to APM proteins showing detectable amounts of antibody at 1:81,000 dilution. And antisera showed much stronger reactivity to APM proteins than any other tissue plasma membrane proteins. Furthermore, tissue specificity of antisera against APM was reconfirmed by immunoblotting using anti-sheep immunoglobulin G-horseradish peroxidase conjugate as a secondary antibody. Antisera to APM proteins showed adipocyte specificity compared with other tissues. In conclusion, polyclonal antibody against APM proteins isolated from pig was developed successfully in our laboratory, and these antisera showed tissue specificity with APM.





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Development of Polyclonal Antibody to Adipocyte Plsma Membrane Proteins Isolated from Korean Native Cattle

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ABSTRACT

The Objectives of this study were to develop polyclonal antibody to adipocyte plasma membrane(APM) proteins isolated from Korean native cattle, and to investigate its tissue specificity. Plasma membrane proteins from adipocyte, liver, heart, spleen, and kidney were isolated using a self-forming percoll gradient. Sheep(40~50kg) was immunized three times at three week interval with the purified APM proteins. Blood was taken from immunized sheep at 10(AS-1), 12(AS-2), and 14(AS-3) days after the third immunization. Antisera titers and cross-reactivity against other tissues were determined by enzyme-linked immunosorbent assay(ELISA). Antisera reacted strongly to the APM proteins showing detectable amounts of antibody at 1:81,000 dilution. The antisera reacted to APM proteins specifically whereas they showed no reactivity to any other tissue plasma membrane proteins. Futhermore, tissue specificity of antisera against APM proteins was reconfirmed by immunoblotting using anti-sheep immunoglobulin G-peroxidase conjugate as a second antibody. The antisera to APM proteins showed adipocyte specificity compared to other tissues. In conclusion, polyclonal antibody against APM proteins isolated from Korean native cattle was development successfully, and these antisera showed the specific reactivity with APM proteins.