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Preparation of Two Kinds of Gene Isoforms of Recombinant Human Serum Paraoxonase-1 and its Specificity for Substrate of Organophosphorus Compounds

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OBJECTIVE: The aim was to establish a new eukaryotic expression system for PON1 that acquires a subtype of a gene on a large scale.

METHOD: We genetically engineered the 192 sites of human paraoxonase-1 to genetically improve the two recombinantly active recombinants using the baculovirus-insect cell expression system. Paraoxonase-1 PON1 192R/Q gene isoform isoenzyme, and then selected three organophosphorus compounds commonly used in agriculture, chlorpyrifos, dioxazine, trisulfide as a substrate.

RESULTS: The PON1 192R/Q subtype isoenzyme was successfully expressed in large quantities using the insect baculovirus protein expression system, and the highly purified target protein was obtained. The PON1 192R/Q gene subtype was mediated by baculovirus to insect cells, and the two subtype isozymes of 192 locus polymorphism of PON1 were successfully expressed. The enzymatic activity of rhPON1 R192 reached 152.5U/mL, the yield reached 8.5%, and the purification reached 435 times. The enzymatic activity of rhPON1 Q192 also reached 74.1U/L, and the enzyme yield reached 12.5%, and the purification reached 597 times. We found that the same quality rhPON1 R/Q192 showed different hydrolysis activities for different organophosphorus compounds such as chlorpyrifos, diazinon, trithiophos and other organophosphorus pesticides, and the hydrolysis activity to chlorpyrifos was rhPON1 R192> rhPON1 Q192; and the hydrolysis activity of diazinon is rhPON1 R192< rhPON1 Q192; for trithiophos, the hydrolysis effects of rhPON1 R192 and rhPON1 Q192 are not good. It is indicated that the polymorphism of 192 locus of PON1 gene may be the main site affecting its activity and causing the difference in substrate specificity of different organophosphorus compounds.

CONCLUSION: This gene polymorphism leads to the change of the primary structure of PON1 protein. Thereby affecting the quaternary structure of the protein, the PON1 192R/Q gene subtypes have different hydrolysis activities on different substrates.