

## Directed evolution and mechanistic studies of serum paraoxonase

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Serum paraoxonase (PON1) is a high density lipoprotein (HDL)-associated enzyme that exhibits a wide range of hydrolytic activities and important anti-atherogenic properties. We have generated recombinant PON1 variants by directed evolution. These enabled the bacterial expression of highly purified PON1, the genetic engineering of new PON1 variants, and the determination of PON1's 3D structure, the mode of its HDL anchoring, and catalytic mechanism. Our structure-reactivity studies and laboratory evolution experiments indicated that PON1's native activity is lactonase. Impairing the lactonase activity through mutations of PON1's catalytic dyad diminished its ability to prevent lipid oxidation and stimulate macrophage cholesterol efflux. Studies of PON1's interaction with reconstituted HDL particles indicated that PON1 is an interfacially activated enzyme. Indeed, apoA-I-HDL binds PON1 with high affinity ( $K_d < 10^{-9}$  M), induces a single highly stable conformation of the enzyme, and selectively stimulates its lactonase activity. These findings enabled the development of new sera tests for the levels and stability of PON1-HDL complex and its lipo-lactonase activity, and opened the road for the engineering of recombinant PON1-HDL particles with therapeutic potential.