Short Communication

Inactivation of Glutamate Racemase of *Pediococcus pentosaceus* with L-Serine O-Sulfate

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Glutamate racemase of *Pediococcus pentosaceus* catalyzes the α,β-elimination of L-serine O-sulfate to produce a pyruvate concomitantly with an irreversible inactivation of the enzyme. α,β-Elimination and inactivation reactions proceed through a common intermediate. L-Serine O-sulfate serves as a suicide substrate of the enzyme.

Glutamate racemase (EC 5.1.1.3) catalyzes the racemization of L- and D-glutamate, and is independent of enzymes such as pyridoxal 5'-phosphate (PLP) and metals.1,2 The racemase is considered to provide almost all bacteria with D-glutamate, which is an essential component of the peptidoglycan layer of the bacterial cell wall,3 and accordingly can be a target for development of new antibiotics. We purified the enzyme from *Pediococcus pentosaceus*4 and cloned its gene into *Escherichia coli*1,2 to construct an overproducer.5 We here report that L-serine O-sulfate, an analog of L-glutamate, serves as a suicide substrate to inactivate the enzyme. This is the first example showing that L-serine O-sulfate acts as a suicide substrate of PLP-independent enzymes.

Glutamate racemase was purified from cells of an *E. coli* clone that harbored pICR223 encoding the enzyme gene.5 L-Serine O-sulfate was synthesized by the procedure of Tubdall.6

Glutamate racemase was inactivated with L-serine O-sulfate as follows. Glutamate racemase (0.4 mg) was incubated with 20 mM L-serine O-sulfate in 1.0 ml of 700 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 8.0) at 37°C. At the time indicated, 50 μl of the mixture was withdrawn, and glutamate racemase was assayed with a coupled system with NAD*+ and glutamate dehydrogenase as described previously.5 A semi-logarithmic plot of the residual activity against time indicated that the enzyme was inactivated with pseudo-first order kinetics (Fig. 1A). Inactivation of the enzyme was also demonstrated by a decrease in the rate of pyruvate formation from L-serine O-sulfate as follows. The reaction mixture contained 700 mM Tris-HCl buffer (pH 8.0), 0.4 mM NADH, 5 units of lactate dehydrogenase, 18 μg of glutamate racemase, and various concentrations of L-serine O-sulfate. The reaction was started by addition of L-serine O-sulfate and a decrease in the absorbance at 340 nm was measured at 37°C with a Shimadzu MPS-2000 spectrophotometer. A decrease in the absorbance at 340 nm, i.e., formation of NAD*+, suggests that the enzyme catalyzes the α,β-elimination of L-serine O-sulfate to produce pyruvate (Fig. 1B). A decrease in the slope of the progress curve indicates the inactivation of enzyme. The pseudo-first order rate constant for the inactivation, k, was obtained from a Guggenhein plot of the data in Fig. 1B with the equation, \( k = 0.693/t_{1/2} \), where \( t_{1/2} \) (in minutes) denotes the time required for the inactivation of one-half of the enzyme initially present. The Michaelis constant of glutamate racemase for L-serine O-sulfate in the α,β-elimination reaction was calculated to be 83.3 mM from the double reciprocal plot of initial rate of the pyruvate formation and the concentration of L-serine O-sulfate (Fig. 2). The value was the same as that of the corresponding constant in the inactivation reaction of the enzyme calculated from the double reciprocal plot of k against the concentration of L-serine O-sulfate (Fig. 2). Identity of the values for both reactions suggests that α,β-elimination of L-serine O-sulfate and inactivation of the enzyme proceeds through a common

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Fig. 1. Reaction of Glutamate Racemase with L-Serine O-Sulfate. (A) Decrease of glutamate racemase activity in the presence (●) or absence (○) of L-serine O-sulfate. Ratio of the residual activity was plotted semi-logarithmically against time. (B) Course of pyruvate formation from L-serine O-sulfate.

Fig. 2. Double Reciprocal Plots of the Initial Rate of the Formation of Pyruvate from L-Serine O-Sulfate (●) and the Rate Constant for Inactivation (○) against the Concentration of L-Serine O-Sulfate.

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Suicide Substrate of Glutamate Racemase

Scheme  A Possible Mechanism for the Inactivation of Glutamate Racemase with L-Serine O-Sulfate.

The enzyme was irreversibly inactivated: no activity of the enzyme inactivated with 120 mM L-serine O-sulfate at 37°C for 3 h was restored by dialysis against 700 mM Tris–HCl (pH 8.0) containing 1 mM D,L-glutamate, and 10% glycerol at 5°C for 14 h.

The results obtained in this work indicate that L-serine O-sulfate serves as a suicide substrate of the enzyme. L-Serine O-sulfate is known as a suicide substrate of several PLP-dependent enzymes such as aspartate aminotransferase,8,9 and glutamate decarboxylase.10 These enzymes catalyze the \( \alpha \)-proton abstraction of the L-serine O-sulfate to produce an \( \alpha \)-aminoacrylate intermediate. The inactivation of each enzyme results from nucleophilic attack by the \( \beta \)-carbon of the aminoacrylate on the internal Schiff base of PLP with the active-site lysyl residue.9,10 Glutamate racemase is independent of PLP, and thus the mechanism of its inactivation is different from those of aspartate aminotransferase and glutamate decarboxylase. A possible mechanism for the inactivation of glutamate racemase with L-serine O-sulfate is shown in Scheme. The \( \alpha \)-proton of L-serine O-sulfate (A) is in the first place abstracted by some active residue of glutamate racemase. Our previous results11 and a recent study by Tanner et al.12 indicate that cysteinyl residues are the catalytic base of glutamate racemase. It is likely that the cysteinyl residue(s) (Cys 73 and/or Cys 184) \( \alpha \)-deprotonates L-serine O-sulfate. The following spontaneous elimination of the sulfate group results in the formation of an \( \alpha \)-aminoacrylate intermediate, a Michael acceptor (B). This mainly is spontaneously hydrolyzed to pyruvate and ammonia (C). The inactivation of the enzyme is assumed to result from the formation of (D), in which the \( \alpha \)-aminoacrylate intermediate is covalently bonded to some nucleophile of amino acid residue such as lysine and cysteine (X) at the active site. L-Serine O-sulfate will be a useful tool to study the reaction mechanism of glutamate racemase. We are now studying the detailed mechanism of this suicide substrate reaction with both enantiomers of serine O-sulfate with emphasis on the identification of the amino acid residue that binds with the inactivation.

References