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 coenzymes 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 Tudball.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hydrochloride (Tris-HCl) buffer (pH 8.0) at 37°C. At the time indicated, 50 μl of the mixture was withdrawn, and glutamate racemase was assayed with a coupled system with NAD⁺ and glutamate dehydrogenase as described previously.5 A semi-logarithmic plot of the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

Guggenheim plot of the data in Fig. 1B with the equation, \( k = 0.693/\tau_{1/2} \), where \( \tau_{1/2} \) (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 proceed through a common

![Figure 1](image1.png)

**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 semilogarithmically against time. (B) Course of pyruvate formation from L-serine O-sulfate.

![Figure 2](image2.png)

**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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intermediate. 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, and glutamate decarboxylase. These enzymes catalyze the α-proton abstraction of the L-serine O-sulfate to produce an α-aminoacylate intermediate. The inactivation of each enzyme results from nucleophilic attack by the β-carbon of the aminoacylate on the internal Schiff base of PLP with the active-site lysyl residue. 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 α-proton of L-serine O-sulfate (A) is in the first place abstracted by some

References