Note

Suicide Reaction of Bacterial ω -Amino Acid Transaminases with Ethanolamine *O*-Sulfate

Kazuo YONAHARA and Seizen TOYAMA

Department of Agricultural Chemistry,
University of The Ryukyus,
Nishihara, Okinawa 903-01, Japan

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γ -Aminobutyrate is an inhibitory neurotransmitter in the vertebrate central nervous system.¹⁾ Accordingly it has become increasingly important to obtain detailed knowledge about the enzyme directly involved in the metabolism of the compound *i.e.* γ -aminobutyrate: α -ketoglutarate transaminase (EC 2.6.1.19, GABA-T). Recently a mechanism-based enzyme inactivator has been developed for investigations of the catalytic mechanism and the physiological function of the enzyme.²⁾ The inactivator has a structural analogy with the substrate and is inactive in itself. In the process of catalytic turn-over, however, a reactive intermediate is generated which binds covalently to a functional group of the enzyme or coenzyme. Thus, the enzyme catalyzes its own inactivation; the compound is referred to as a suicide substrate. Ethanolamine *O*-sulfate,³⁾ γ -acetylenic GABA,⁴⁾ γ -vinyl-GABA,⁵⁾ 4-amino-5-halopentanoate,⁶⁾ and the naturally occurring cyclic *m*-

aminocyclohexadiene carboxylate (gabaculine)⁷⁾ are well-known suicide substrates of mammalian GABA-T. We have found a suicide reaction of bacterial ω -amino acid: pyruvate transaminase (EC 2.6.1.18, ω -APT) with gabaculine.⁸⁾ The compound also inhibited bacterial GABA-T.⁹⁾ In this communication we describe a further example of suicide reaction of bacterial ω -amino acid transaminases with ethanolamine *O*-sulfate. Taurine: α -ketoglutarate transaminase (EC 2.6.1.55), ω -APT, and GABA-T are employed for the target enzyme, because the compound is a substrate analogue for the first enzyme *i.e.* taurine, and the latter two enzymes are the principal enzymes involved in the metabolism of β -alanine and γ -aminobutyrate in bacteria.

Ethanolamine *O*-sulfate was purchased from Nakarai Chemicals Co., Kyoto. Taurine transaminase was purified from *Achromobacter superficialis*,¹⁰⁾ and ω -APT¹¹⁾ and GABA-T⁹⁾ were from *Pseudomonas* sp. F-126 as described previously. The inactivation of the ω -amino acid transaminases by ethanolamine *O*-sulfate was measured by incubating the enzyme and the inhibitor at 37°C. Under the conditions employed, with no inhibitor present, no loss of the enzyme activity occurred during the course of the experiment.

Ethanolamine *O*-sulfate is a non- or less-reactive substrate of the bacterial ω -amino acid transaminases,^{9~11)} but a competitive inhibitor against the amino donor of these enzymes. The K_i values obtained by a Lineweaver-Burk plot are 0.014, 0.66, and 17.2 mM for taurine transaminase, ω -APT, and GABA-T, respectively. When the transaminases were incubated with the inhibitor in the absence of an amino donor, the enzymes were inactivated. The inactivation process followed pseudo-first

FIG. 1. Time Dependent Inactivation of Bacterial ω -Amino Acid Transaminases with Ethanolamine *O*-Sulfate.

The enzyme was incubated with ethanolamine *O*-sulfate in the presence or the absence (dashed line) of an amino acceptor at 37°C for the indicated time, and then the remaining activity was determined. The incubation mixture contained 100 μ M potassium phosphate buffer (pH 8.0), 20 μ M amino acceptor, 0.1 μ M pyridoxal 5'-phosphate, the enzyme (0.02 unit) and the inhibitor in a final volume of 0.9 ml. The inhibition was terminated by the addition of an amino donor (20 μ M in 0.1 ml), and the enzyme activity was assayed by determining malonic semialdehyde or succinic semialdehyde with *o*-aminobenzaldehyde.^{9,11)} Amino acceptor: α -ketoglutarate for taurine: α -ketoglutarate transaminase (A) and γ -aminobutyrate: α -ketoglutarate transaminase (C), and pyruvate for ω -amino acid: pyruvate transaminase (B). Amino donor: β -alanine for taurine transaminase and ω -amino acid: pyruvate transaminase, and γ -aminobutyrate for γ -aminobutyrate transaminase. Concentration of ethanolamine *O*-sulfate: (A): 1 and 3; 50 μ M, 2; 20 μ M. (B): 1 and 3; 0.5 mM, 2; 0.2 mM. (C): 1 and 3; 5.0 mM, 2; 2.0 mM. 1: Pyridoxamine 5'-phosphate form of the enzymes.

order kinetics as observed for mammalian GABA-T³⁾ (Fig. 1). Amino acceptors (pyruvate or α -ketoglutarate) had no effect on the inactivation. The enzyme inactivated could not be reactivated either by dialysis or in the presence of pyridoxal 5'-phosphate (pyridoxal-P). The enzymes were incubated with an amino donor to convert enzyme-bound pyridoxal-P to pyridoxamine 5'-phosphate (pyridoxamine-P), and then the inactivation was examined with ethanolamine O-sulfate as described for the pyridoxal-P form of the enzymes. The pyridoxamine-P enzymes, however, were not substantially inactivated, indicating that the inhibitor itself does not react with the enzymes. These results suggest that ethanolamine O-sulfate binds to the active site of the enzymes and the inactivation is caused by a reactive intermediate formed during the enzyme catalysis: suicide reaction. In the case of mammalian GABA-T an active β -aminoethylene was suggested for the possible intermediate, which was formed by a β -elimination of the inhibitor catalyzed by the enzyme.³⁾ The results presented here, together with that obtained with mammalian GABA-T, also suggest a common structure of the active site or a catalytic mechanism of ω -amino acid transaminases.

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