SPECIFIC BINDING OF β-LACTONE 1233A TO 3-HYDROXY-3-METHYLGLUTARYL-COEZYME A SYNTHASE

Sir:

A naturally occurring β-lactone, 1233A (F-244, L-659,699), the structure of which has been elucidated to be 2R-hydroxymethyl-3R-hydroxy-8R,10,12-trimethyl-(E,E)-10,12-tetradecadienoic acid 1,3-lactone,1,2) is a potent and specific inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase.3~5) Several research groups have studied the mode of action of 1233A on HMG-CoA synthase, but the reversibility of 1233A inhibition was still unclear. We reported preliminarily that the inhibition of HMG-CoA synthase by 1233A is irreversible because the enzyme activity was not recovered by any degree of dilution when the enzyme was pretreated with 1233A.4) However, by similar experiments, Greenspan et al.5) reported the data suggesting reversible inhibition, while Mayer et al.6) suggested irreversible inhibition. To clarify this discrepancy, we tried to use [14C]1233A with a highly specific radioactivity prepared biosynthetically.7) In this communication, we provide the evidence showing that 1233A inhibits HMG-CoA synthase by binding to the enzyme specifically and irreversibly.

The preparation of 1233A and [14C]1233A (27.2 Ci/mol) was reported previously.7,8) HMG-CoA synthase was prepared from livers of rats fed lovastatin (0.1%) and cholestyramine (5%) for 7 days. The enzyme was purified through the DEAE-cellulose step according to the similar method of Mehrabian et al.9) HMG-CoA synthase activity was assayed by two methods. In Method A, the breakdown of acetoacetyl-CoA in the presence of acetyl-CoA or CoA was followed by monitoring spectrophotometrically at 303 nm of the enol form as described by Lowe and Tubbs.10) The assay mixture containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 10 μM acetoacetyl-CoA and enzyme in a total volume of 950 μl was preincubated at 30°C and the reaction was initiated by the addition of 50 μl of 2 mM acetyl-CoA (final 100 μM). In Method B, HMG-CoA synthase was assayed using [1-14C]acetyl-CoA as described previously.4)

The result given in Fig. 1 showed the irreversible inhibition of HMG-CoA synthase by 1233A. The IC50 value in the experiment without preincubation was calculated to be 0.65 μM, supporting the previous observation (IC50: 0.20 μM).4) However, when the enzyme was preincubated with 0.5 μM 1233A which produced only 35% inhibition in the assay without preincubation, no enzyme activity was observed regardless of the final concentration of 1233A (0.05~4.0 μM). Furthermore, even after Sephadex G-25 gel filtration or dialysis of the pretreated enzyme, no HMG-CoA synthase activity was recovered (data not shown).

To examine whether 1233A reacts directly on HMG-CoA synthase, partially-purified HMG-CoA synthase was incubated with [14C]1233A at 37°C for 5 minutes, and the reaction was stopped by heating the mixture in the presence of SDS. The SDS-PAGE analysis revealed that only 53 kilodalton protein was labeled with [14C]1233A (Fig. 2), suggesting that 1233A reacts specifically with the subunit of HMG-CoA synthase.9,11) The [14C]-1233A-bound protein was resistant to SDS-, heat-, and mercaptoethanol-treatments. The β-lactone ring of 1233A, responsible for HMG-CoA synthase inhibition, appears reactive with a cysteine SH-group, or a serine or threonine OH-group. In fact, Hadvary et al.12) demonstrated that β-lactone tetrahydrolipstatin blocks pancreatic lipase by binding covalently to the active site serine. From the study on the reaction mechanism of HMG-CoA synthase, it is proposed that the active site cysteine SH-group is rapidly acetylated by acetyl-CoA to yield acetyl-S-enzyme and that further condensation reaction with acetoacetyl-CoA is proceeded on...
HMG-CoA synthase (500 μg) was incubated with [14C]1233A (4 nmol, 0.11 μCi) at 37°C for 5 minutes in a total volume of 100 μl. Then, 100 μl of a buffer containing 6.25 mM Tris-HCl (pH 6.8), 2% (w/v) SDS, 10% glycerol, 5% (v/v) 2-mercaptoethanol and 0.001% BPB was added and the mixture was heated at 100°C for 3 minutes. Twenty microliters of the sample was applied for electrophoresis.

the enzyme to form HMG-CoA.\textsuperscript{13,14} Therefore, it is possible to imagine that the carbonyl moiety of the β-lactone ring attacks the active site cysteine to form an enzyme bound thioester. Miziorko et al.\textsuperscript{15} reported that 3-chloropropionyl-CoA inhibits irreversibly HMG-CoA synthase by alkylating the active site cysteine and that the synthase activity was protected against the inactivation by the inhibitor when it was preincubated with the substrate, acetyl-CoA or acetoacetyl-CoA. However, the inhibition caused by 1233A was not prevented even when the synthase was preincubated with 100 μM acetyl-CoA or acetoacetyl-CoA. It might be that the reactivity of 1233A to the cysteine is much higher than that of the substrates or that 1233A alkylates an amino acid of unknown active sites other than the active site cysteine.

As shown in Fig. 3, a linear correlation between [14C]1233A bound to the enzyme and the enzyme inhibition was shown. In this experiment, about $1 \times 10^4$ dpm of [14C]1233A (170 pmol) bound to the enzyme produced the complete inhibition of HMG-CoA synthase. Based on the purity of the synthase estimated from the specific enzyme activity in comparison with that of the homogenous synthase, it is speculated that one to two moles of 1233A are bound to one mole of the enzyme with complete loss of the enzyme activity. This appears reasonable because HMG-CoA synthase comprises homo dimers of the 53 kilodalton subunit.\textsuperscript{9,11} However, the binding site and the binding mode of 1233A remain to be investigated.

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