RESEARCH ON THE CORRELATION BETWEEN THE PUMMERER REACTION AND PENICILLIN BIOSYNTHESIS
(REVIEW)

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Research on the correlation between the Pummerer reaction mechanism and β-lactam formation during penicillin biosynthesis is discussed based on our silicon-induced asymmetric Pummerer type reaction.

INTRODUCTION

During the biosynthesis of penicillin, when a precursory tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) is converted to isopenicillin N in both fungi and bacteria, one of the hydrogen atoms at C-(3) of the cysteine residue of ACV is perfectly discriminated by removing the enzyme isopenicillin N synthase (IPNS) to form a β-lactam ring which has 5,6-cis stereochemistry. How can such a complete stereoselection be achieved by the enzyme in vivo? To answer this question, we have started model studies for the biosynthesis of penicillin using the Pummerer type reaction of sulfoxides. Despite our many experiments carried out in vitro for this purpose, and some interesting results have already been reported, the accurate mechanism of β-lactam ring formation is still obscure. However, just recently, Professor Baldwin has finally solved the question by X-ray crystallographic analysis of IPNS in the presence of Fe(II) and ACV [1], in addition to the information gained from their vast substrate analog studies. The unquestionable chemical mechanism of IPNS has been answered. Their findings have put to rest the problem which has long been full of mystery.

We have been carrying out the development of the asymmetric Pummerer reaction [2] in parallel with the biomimetic synthesis of penicillin using the silicon-induced Pummerer reaction as a key step. Prior to the publication of this paper, we have carried out partial research studies. Here, we summarize our project on penicillin studies in more detail centering on our silicon-induced Pummerer type reaction, since we feel that it is time to wind up the project.

PENDICILLIN BIOSYNTHESIS

A key step in the biosynthesis of penicillin is the cyclization of ACV to isopenicillin N, catalyzed by the iron-dependent enzyme IPNS (Scheme 1). The mechanism of the reaction has no counterpart in the standard repertoire of organic chemical reactions, proceeding by stereoselective removal of four hydrogen atoms from the substrate [3].

The investigation of the biosynthesis of penicillin was started for the purpose of introducing unnatural side chains into penicillins in the 1940s [4]. The pathway of penicillin biosynthesis exhibits several interesting chemical reactions, especially β-lactam ring formation, thiazolidine ring formation, and ring expansion from penicillins to cephalosporins, with which many scientists have been fascinated. Revealing the intimate details of the ring closures has been the main subject in investigations of penicillin biosynthesis. Despite extensive investigation by many biochemists, organic chemists, and inorganic chemists, even the sequence of ring closure could not be elucidated. This
was apparently due to the fact that the detection of the intermediates on the pathway from ACV to isopenicillin N had never been successful. Although it seemed impossible to experimentally establish the chemical mechanism, a powerful study for the elucidation of penicillin biosynthesis by Baldwin, with his theory based upon a bold hypothesis and with excellent enzymatic techniques, has gradually thrown light on it [5]. He and his co-workers probed the fact that the β-lactam ring is formed first by using isotopic competition experiments [6]. Furthermore, the mechanism of the second ring closure, i.e., thiazolidine ring formation, was explained in terms of his novel and unconventional idea that the participation of highly reactive ferryl species in the reaction is a key feature of IPNS [5]. They even demonstrated that the second ring closure may be effected in a biomimetic way in the absence of the enzyme, by treatment of a β-lactam thiol with oxygen, iron(II), and appropriate co-factors (Scheme 2) [7]. In spite of their great efforts, the first step in the biosynthesis of penicillin, β-lactam formation, was still a fascinating mechanistic problem.

Historically, several mechanisms have been proposed [3]. Earlier, two hypothetical intermediates involving 2,3-dehydrocysteinyl structures A, B were ruled out by labelling experiments. Other proposed intermediates including thiazepine C [8] and hydroxamic acid D [9] were discredited based on the results of experiments on transformation of ACV analog into penicillin under incubation conditions. Two mechanisms called the activated alcohols E theory [3] and thioaldehyde F theory [10] still remain because no negative results against them have been reported.