Frontiers of Antibiotic Research

Edited by

Hamao Umezawa

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Foreword

The Takeda Science Foundation was established in 1963 by the late Mr. Chobei Takeda VI with an endowment from Takeda Chemical Industries, Ltd. Since then, the Foundation has contributed to the advancement of science and technology, and other cultural affairs throughout the world as well as in Japan. This has been done by awarding prizes to recognize outstanding research achievements in medical science, giving grants to researchers engaged in science and technology, providing fellowships to medical scientists studying in Japan, and preserving reference books and materials on traditional Asian medical science, which are made available to the public at the "Kyoou Shooku" library.

In addition to the above activities, the Takeda Science Foundation holds International Symposiums on Bioscience. The members of the Symposium Committee are: Setsuro Ebashi, M.D. (Professor Emeritus, University of Tokyo; Professor, National Institute of Physiological Sciences); Osamu Hayaishi, M.D. (Professor Emeritus, Kyoto University; President, Osaka Medical College); Tomoji Suzuki, Ph.D. (Professor Emeritus, Kyoto University); Hamao Umezawa, M.D. (Professor Emeritus, University of Tokyo; Director, Institute of Microbial Chemistry); and Yuichi Yamamura, M.D. (Professor Emeritus, Osaka University).

The first Takeda Science Foundation Symposium on Bioscience was held in 1982, and every Symposium has been very successful. The fourth Symposium entitled "Frontiers of Antibiotic Research", held in Kyoto from November 25th to 27th, 1986, was organized by Dr. Hamao Umezawa, the chairman. The proceedings of this Symposium were edited for publication by the Organizing Committee.

Regretfully, however, Dr. Hamao Umezawa who was so dedicated to the accomplishment of this Symposium, passed away on December 25th, 1986, just one month after the Symposium.

This Symposium proved to be a great success and has contributed to the progress of bioscience by providing a forum for international exchange of scientific knowledge and experience. I hope that together the Symposium and proceedings will be a tribute to Dr. Umezawa and to his brilliant research career and dedication to the field of antibiotics.

I would like to express my sincere gratitude to the members of the Symposium Committee for supervising the fourth Symposium, and to the members of the Organizing Committee for planning such a wonderful program and helping to make the symposium a success.

Einosuke Ohmura, Ph.D.
Chairman, the Board of Trustees
Takeda Science Foundation

Preface

Antibiotic research covers a wide range of fields from basic to clinical investigations. Professor Hamao Umezawa, the former Director of the Institute of Microbial Chemistry, in Tokyo, was a powerful leader in the field of antibiotic research due to his wide range of knowledge in all these fields. Because of this, he was asked by the Takeda Science Foundation to chair the organizing committee for the symposium, "Frontiers of Antibiotic Research", which was held on November 25th ~ 27th, 1986.

Before receiving this request, he had intended to organize a symposium on antibiotic research for the 25th anniversary of the Institute in 1987. With this in mind, he placed special emphasis on ß-lactam antibiotics for the present symposium. The aim was to provide up-to-date information on the recent research on ß-lactam and associated antibiotics, including action, resistance, pharmacokinetics, inhibitors of ß-lactamase and cell wall synthesis, biosynthesis, chemical synthesis and computer-assisted drug designing.

The symposium was held in accordance with his plans and was a great success. The high caliber of the contributions from the invited speakers and participants is reflected in these Proceedings. We believe this book will be of benefit to all researchers in areas of antibiotic research.

Unfortunately, Professor Umezawa was unable to attend the symposium because of serious illness. He passed away on December 25th, 1986, a month after the symposium. The members of the Editorial Board have attempted to complete his job in keeping with his ideas.

MEMBERS OF THE ORGANIZING COMMITTEE & EDITORIAL BOARD.

Proceedings of the Fourth Takeda Foundation Symposium on Bioscience — 1986

Frontiers of Antibiotic Research

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VIII. Theoretical Drug Designing

I. Biosynthesis and Chemistry of β-Lactams

RECENT STUDIES ON THE BIOSYNTHESIS OF PENICILLINS AND CEPHALOSPORINS

Jack E Baldwin

The Dyson Perrins Laboratory
University of Oxford
Oxford

In the first part of this lecture I would like to review the earlier work we have carried out on the biosynthesis of penicillin since I believe that the nature of this step is crucial to understanding the subsequent conversion of penicillin into cephalosporin, which is the objective of our most recent studies.

We have described the discovery in C. acremonium of an enzyme, isopenicillin N synthase (IPNS), which catalyses the conversion of tripeptide 1 into isopenicillin N 2, concomitant with the reduction of one molecule of dioxygen, fig.1 (1-2). Only those hydrogen atoms circled in this

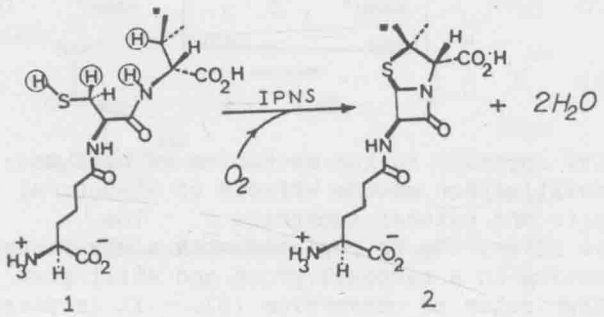

Fig 1

figure are removed from the substrate (3) and labelling studies have shown that that both new bonds, C-N and C-S are formed with retention of configuration (4-5). Thus the enzyme IPNS is acting as a desaturase ie removing

hydrogen from the substrate to form the ring structures of the product. This enzyme was purified in Oxford in collaboration with Professor Sir Edward Abraham in the William Dunn School, and an N-terminal sequence determined (6) and the gene isolated and sequenced by use of DNA probes based on the N-terminal sequence (7). The gene corresponds to an enzyme of M_r 38,000 containing two cysteines. No unusual cofactors appear to be involved but the enzyme is associated with iron and preliminary studies indicate a ratio of 1 atom of iron/mole enzyme. Ascorbate is essential to maintain enzyme activity. The gene has been cloned into a strain of E. coli and the expressed recombinant enzyme (ca 20% of cell protein) behaves in all respects identically to the natural fungal enzyme. Studies of the kinetic isotope effects of deuterium substitution into precursor 1 have been interpreted on the basis of a two step mechanism involving first loss of the β-cysteinyl hydrogen, followed by loss of the β-valinyl hydrogen. These studies also indicate that no enzyme free intermediate is involved. Consequently we believe that the simplest explanation of the isotope effects is that an enzyme-bound monocyclic lactam 3 is the intermediate, fig. 2 (8). In

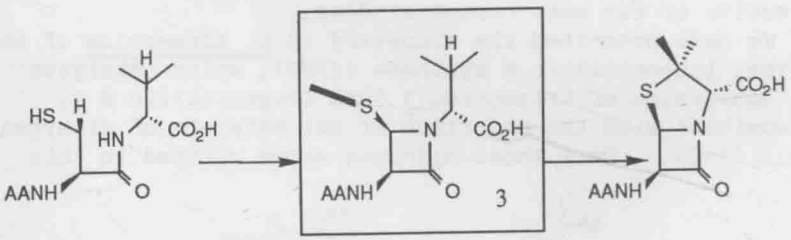

fig 2

an alternative approach to the mechanism we have made an extensive investigation on the effects of structural modification to the natural substrate 1. The α-aminoadipyl moiety can be replaced with a six carbon chain terminating in a carboxyl group and still give relatively high rates of conversion (9). It is possible to make penicillin V and penicillin G from the corresponding dipeptides but the rates of conversion, in the absence of the carboxyl group, are very low (10). The cysteinyl moiety can be changed and yet still permit penicillin synthesis by the substitution of methyl groups for α- and β (pro-R) hydrogen atoms but the β-(pro-S), site will not tolerate substitution, since this is the hydrogen

normally removed in the enzymic step, fig. 3. Structural

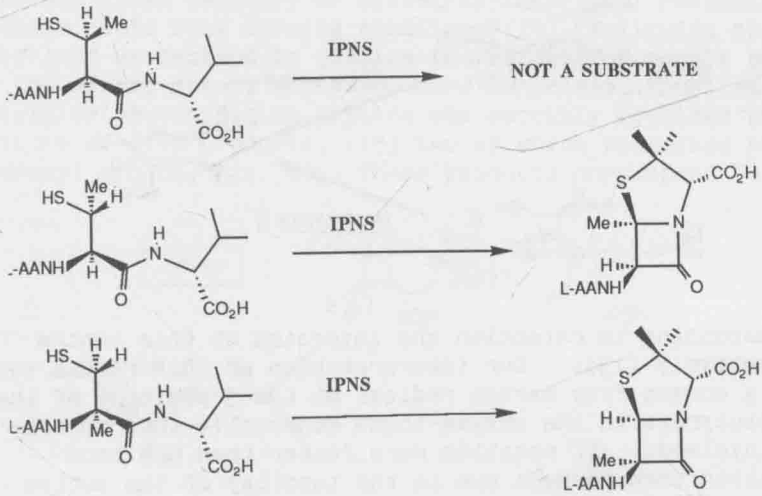

fig 3

variation of the valine portion has led to some very interesting and surprising results. Thus the α-aminobutyrate analogue was found to give simultaneously three products, two penicillins and a cepham in the ratios shown, fig.4 (11). Intriguingly a group at ICI have found

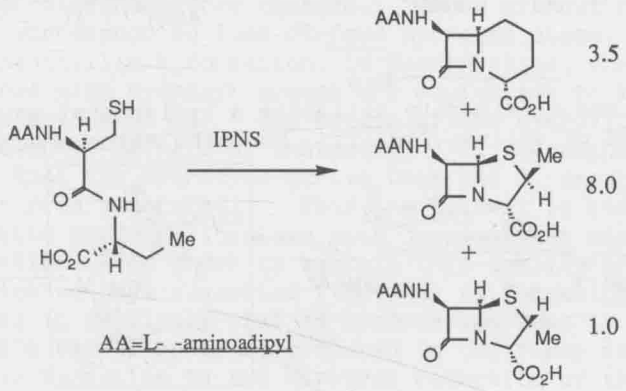

fig 4

the two major products, as their D-α-aminoadipyl derivatives, in certain Streptomyces (12). Even more unusual was the finding that when the β-hydrogens of the aminobutyrate moiety were stereospecifically replaced by deuterium both the 3R and 3S monodeuterated peptides were