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특별강연초록 (I)

Microbial Degradation and Enzymes Active on Nylon Oligomers

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Microbial degradation of unnatural synthetic substances are interesting from hypothesis that a new metabolic pathway should be established from the unnatural compound to a common metabolic intermediate fro such an ability. The establishment of a new pathway essentially require a creature of new enzyme active on the unnatural synthetic compound which have never existed on the earth. One of such unnatural synthetic compounds is nylon oligomers. We isolated a bacteria capable to grow on a medium containing 6-aminohexanoic acid cyclic dimer (Hxa-c-dimer) as the sole carbon and nitrogen sources, and determined to be *Achromobacter guttatus* K172. The key enzymes responsible to Hxa-c-dimer metabolism were purified to homogeneous state and their characteristics were assayed.

Two enzymes were found to be responsible for the conversion of Hxa-c-dimer to 6-aminohexanoic acid (Hxa), one is Hxa-c-dimer hydrolase which hydrolyze Hxa-c-dimer to Hxa-linear dimer(Hxa_2) the other enzyme is Hxa oligomer hydrolase (nylon oligomer hydrolase) which converts Hxa oligomers to the monomer. So, the reaction sequence was estimated as follow:

Hxa-c-dimer——Hxa_2——2 Hxa.

Hxa-c-dimer hydrolase was purified from crude cell extract after first and second DEAE-Sephadex column chromatography and a molecule shieve column chromatography on Sephadex G-200. The purified enzyme had a molecular weight of 110,000 obtained by column chromatography on G-200 and by ultracentrifugation, and its subunit had a molecular weight of 55,000 analyzed by SDS-polyacrylamide gel electrophoresis. So, the enzyme was estimated to be a dimeric enzyme of which subunit has a molecular weight of 55,000.

This enzyme had no activity on natural substrates including, 55 kinds of dipeptide, 16 kinds of tripeptides, oligopeptides, lactams, and lactam antibiotics such as penicillin and cepharospolin. The failure to find out a natural substrate of this enzyme may mean that the enzyme had been modified seriously to adapt new substrate by mutation and lost its original ability.

Nylon oligomer hydrolase was purified by DEAE-Sephadex A 50 column chromatography, preparative disc gel electrophoresis and molecular shieve column chromatography on Sephadex G-200. The best sample obtained showed a single protein band by SDS-polyacrylamide gel disc electrophoresis. The molecular weight estimated by column chromatography on Sephadex G-200 was 84,000 and that of its subunit analyzed by SDS-polyacrylamide gel electrophoresis was 42,000. So, it can be concluded that nylon oligomer hydrolase is a dimeric enzyme of which subunit has 42,000 daltons in its molecular weight.

This enzyme is active on various Hxa oligomers of less than 20 in polymerization number. Hxa_{100} was not hydrolyzed. The enzymer eact with Hxa oligomers as an exohydrolase; liberate Hxa monomer from the N terminal stepwisely.

This enzyme does not hydrolyze 55 kinds of dipeptides tested but it had a slight activity of dipeptidyl aminopeptidase activity and active on

peptides to liberate dipeptide from the N-terminal.

From these results the original enzyme from which nylon oligomer hydrolase was derived was estimated.

특 별 강 연 초 록 (Ⅱ)

Some Problems in New Antibiotic Research

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It is certainly a difficult work to find out novel-type "practical" antibiotic from natural sources. In fact, chemically modified antibiotics, such as synthetic penicillins, cephalosporins, derivatives of tetracycline, lincomycin, rifamycin and kanamycin are most widely used in medical practice at present. However, as the possibility of success gained by chemical modification is limited, it would be the buty of our applied microbiologists to offer novel antibiotics to chemist and physician without interruption. In this lecture, some practical problems in new antibiotic reserch will be discussed.

1, Object of Screening

At the beginning of screening for new antibiotic, it is important to determine the main purpose of the programme. As the majority of the workers in this field is occupied by employees of pharmaceutical companies, estimation of screening object shown in Table is made on the view point of industrial researchers. If the screening is planned in public or academic institute, some different estimation would be made in the Table.

2. Screening Process

The ordinary process used for screening new microbial product from natural sources is schematically shown in Figure. The organism which plays most important role in antibiotic producer is actinomycetes. Soil samples are usually used for isolation of microorganisms. It can be said that new product is generally obtained from cultures of freshly isolated organisms. However, as the new isolate apt to diminsh its special activity rapidly, it is important to keep the novel ability by successive selection for superior strain. A key to success in new antibiotic screening would be to abandon known or uninteresting candidate as early as possible. For doing this selection satisfactorily, accumulation of wide experience and ceaseless documentation of new microbial substance are primarily necessary.

3. Recent Trends in Screening for New Microbial Products.

(1) Search for now microbial origin: Most of the antibiotic producer belongs to *Streptomyces* species. However, other genus of *Actinomycetes* namely, *Micromonospora*, *Nocardia* or *Actinoplanes* have become the objects of new antibiotic screening. *Basidiomycetes*, *Pseudomonas* related bacteria and marine microorganisms also attract interests of many workers. Studies on nutritional mutants bring artificial new antibiotic such as hybrimycin from neomycin producing strain.

2) Control of cultural conditions: New antibiotics can often be found out by modifying customary cultural conditions. The most succssful example was achieved by Imanaka. He added unordinary much phosphate to medium and kept the pH value slightly acidic throughout the fermentation. Using this process, he could discover many novel type antibiotic such as pyrrolnitrin, thiopeptin and bicyclomycin. Some examples can also be mentioned cryomycin was discovered by Ogata under low temperature cultivation; mimosamycin and chlorocarcias were isolated by Arai from vigorously aerated culture of streptothricin producing *St. lavendulae*.

3) Trials of new screening methods: Varieties of new or reformed screening methods were tried to obtain novel products from microbial sources. They would be divided into the four categories; 1) using modified microbe as test organism, 2) using intact animal or plant bodies as physiological test organism, 3) screening for specific enzyme inhibitor, 4) usinh specific chemical reaction (e. g. color test). The striking success in recent antibiotic research would be the discovery of nocardicins by Fujisawa group using β-lactam sensitive mutant as the assay organism. Moreover, some new micrrobial products such as pepstatins, derivatives of fusaric acid screened out as specific enzyme inhibitors became