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Biodegradation of Nylon and other Synthetic Polyamides

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|---------|--|
| Abu | 4-aminobutyric acid |
| Abu-Abu | N-4-aminobutryl-4-aminobutyric acid |
| Abu-Ahx | N-4-aminobutryl-6-aminohexanoic acid |
| Ahx | 6-aminohexanoic acid |
| Ahx-Abu | N-6-aminohexanoyl-4-aminobutyric acid |
| Ahx-Ahx | 6-aminohexanoate-dimer |
| Ahx-Ani | 6-aminohexanoylaniline |
| Ahx-Aoc | N-6-aminohexanoyl-8-aminooctanoic acid |
| Aoc | 8-aminooctanoic acid |
| COD | chemical oxygen demand |
| EI | 6-aminohexanoate-cyclic-dimer hydrolase |
| EII | 6-aminohexanoate-dimer hydrolase |
| EII' | a protein having 88% homology to the EII encoded on the RS-IIB region of pOAD2 |
| EIII | endo-type 6-aminohexanoate-oligomer hydrolase |
| F-EI | EI enzyme from <i>Flavobacterium</i> |
| F-EII | EII enzyme from <i>Flavobacterium</i> |
| F-nylA | EI gene from <i>Flavobacterium</i> |
| F-nylB | EII gene from <i>Flavobacterium</i> |
| kb | kilo-base-pairs |
| nylB' | gene for the EII' protein |
| nylC | gene for the EIII enzyme |
| P-EI | EI enzyme from <i>Pseudomonas</i> |
| P-EII | EII enzyme from <i>Pseudomonas</i> |
| P-nylA | EI gene from <i>Pseudomonas</i> |
| P-nylB | EII gene from <i>Pseudomonas</i> |

1

Introduction

A polyamide is a polymer, which contains recurring amide groups (R-CO-NH-R') as integral parts of the main polymer chain. Synthetic polyamides are produced by a condensation reaction between monomers, in which the linkage of the molecules occurs through the formation of the amide groups. Polyamides may be made from a dicarboxylic acid and a diamine, or from an amino acid or its lactam that is able to undergo self-condensation. One of the most common polyamides, nylon-6,6, is produced by the interaction of hexamethylenediamine and adipic acid. When ϵ -caprolactam is the

starting material, nylon-6, which consists more than 100 units of 6-aminohexanoic acids is obtained. However, during the polymerization reaction, some molecules fail to polymerize and remain as oligomers, while others undergo head-to-tail condensation to form cyclic oligomers. These nylon oligomers are the byproducts from the nylon-6 factories, thereby contributing to the increase in the industrial waste material into the environment.

Recently, biodegradation or detoxification of xenobiotic compounds has been recognized as a useful way to eliminate environmental pollutants. However, the efficiency of removal is highly dependent on the specific enzymes that can catalyze the desired deg-

radation reactions. In this chapter, the historical outline of nylon manufacture, biological degradation of nylon and its oligomers, and adaptation of microorganisms toward these synthetic compounds, will be described.

2

Historical Outline

The most important polyamides are the nylons, which are indispensable materials for fibers and plastics. Nylon-6,6 was developed in the 1930s by Carothers and coworkers, and its commercial production began in 1939 at DuPont's plant in the USA. Soon after the DuPont fiber was marketed, nylon-6 was produced in Europe based on the polymerization of ϵ -caprolactam. Nylon-6 and nylon-6,6 have almost the same structure, and high resistance to wear, heat, and chemicals, and therefore are the important polyamide fibers worldwide, especially in clothing and home furnishings. By varying the acid and the amine, various products which differed in the hardness, toughness, softness and resilience (rubberiness), have been produced. Industrial uses of nylon fiber include automobile tires and ropes, whilst as plastics the nylons are used as an engineering plastic, for example, in bearings and gears.

Following the success of nylons, aromatic nylons (aramids) were prepared by condensation of a diamine and terephthalic acid. The close packing of the aromatic polymer chains generated a strong, high-melting fiber for radial tires, heat-resistant fabrics, and bulletproof clothing, etc. DuPont began to produce Nomex (poly-*m*-phenylene isophthalamide) in 1961 and Kevlar (poly-*p*-phenylene terephthalamide) in 1971. In 1978, Kwolek produced from aramids the first polymeric liquid crystals. Aramids are

not produced in as high a volume as nylons, but because of their high unit price, they represent a large business. End uses for aramids in the home are few, but industrial uses are increasing.

Other polyamides of commercial importance include nylons-4,6; 6,10; 6,12; and 12,12. These are prepared from diamines and dicarboxylic acids. Nylon-11, prepared by step-growth polymerization from the amino acid $\text{H}_2\text{N}(\text{CH}_2)_{10}\text{COOH}$; and nylon-12, made by ring-opening polymerization of a cyclic amide.

3

Chemical Structures, and Preparation of Nylon Oligomers

The chemical structure of nylon and its related compounds are shown in Figure 1. Substrates used for the testing of biodegradation, which contain the same linkages with nylon, but differ in the molecular weight, are also shown. Preparations of these compounds are as follows.

3.1

Cyclic Oligomers of 6-Aminohexanoate

Unfractionated nylon oligomers obtained from nylon-6 factory were dissolved to saturation in hot water. Any insoluble materials were then removed by filtration, the solution was cooled to ca. 5°C, and crystals, which contain the cyclic dimer as major fractions, were obtained (Kinoshita et al., 1975).

The 6-aminohexanoate cyclic oligomers with a higher degree of polymerization were purified from unfractionated nylon oligomers by Sephadex LH-20 gel filtration column equilibrated with methanol (Kakudo et al., 1993).

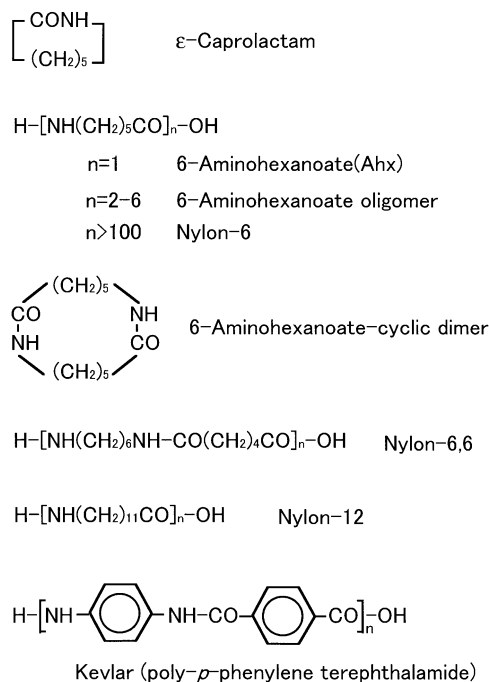


Fig. 1 Structure of nylon and its related compounds.

3.2

Linear Oligomers of 6-Aminohexanoate and their Related Compounds

To obtain the following substrates used for the enzyme reactions, namely 6-aminohexanoate-dimer (Ahx-Ahx), *N*-4-aminobutyryl-4-aminobutyric acid (Abu-Abu), *N*-4-amino-

butyryl-6-aminohexanoic acid (Abu-Ahx), *N*-6-aminohexanoyl-4-aminobutyric acid (Ahx-Abu), *N*-6-aminohexanoyl-8-aminooctanoic acid (Ahx-Aoc), 6-aminohexanoylaniline (Ahx-Ani), *N*-8-aminooctanoyl-6-aminohexanoic acid (Aoc-Ahx), chemical synthesis is necessary. The scheme of chemical synthesis of the amide compound, $\text{H}_2\text{N}(\text{CH}_2)_m\text{CO}-\text{HN}(\text{CH}_2)_n\text{COOH}$, from two amino acids, $\text{H}_2\text{N}(\text{CH}_2)_m\text{COOH}$ and $\text{H}_2\text{N}(\text{CH}_2)_n\text{COOH}$, is shown in Figure 2. Amino groups of amino acids ($\text{H}_2\text{N}(\text{CH}_2)_m\text{COOH}$) are modified by carbobenzoxy chloride, and amide bonds are formed between the modified compounds and the other amino acids ($\text{H}_2\text{N}(\text{CH}_2)_n\text{COOH}$) by mixed anhydride method using ethylchloroformate and trimethylamine in toluene. After purifying the *N*-carbobenzyloxy amide compounds, carbobenzyloxy groups were deprotected by catalytic reduction using Pd/charcoal under hydrogen flow. Linear oligomers of 6-aminohexanoate (from dimer to hexamer) were also chemically synthesized stepwise by repeating the similar procedure (Figure 2) (Fujiyama et al., 1991).

4

Production and Main Applications of Nylon

Nylon is a very important fiber, and its market has grown greatly since its introduc-

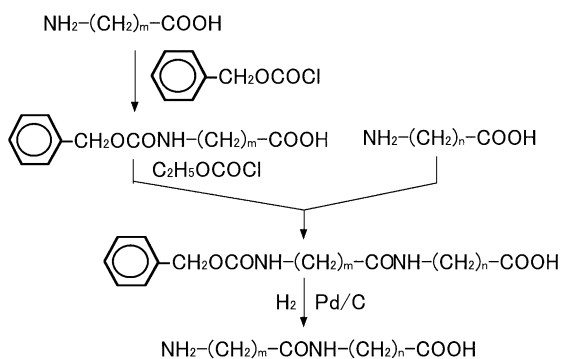


Fig. 2 Chemical synthesis of linear amide compounds from two amino acids, $\text{H}_2\text{N}(\text{CH}_2)_m\text{COOH}$ and $\text{H}_2\text{N}(\text{CH}_2)_n\text{COOH}$.

tion. In 1999, nylon was produced as a filament or staple worldwide, and its total amount of production was 3.9×10^6 ton/year, of which USA and Canada shared ca. 40% (Table 1). Market prices of nylon compounds in USA in 2001 were \$2.84–2.93 per kg (nylon-6), and \$3.09–3.41 per kg (nylon-6,6). The prices for other types of nylon (nylon-6,10, nylon-6,12, nylon-12, etc.) ranged from \$5.5 to \$7.7 per kg. Major nylon-producing companies are listed in Table 2.

The high strength, elasticity, luster, abrasion resistance, dye ability, and shape-holding characteristics of nylon made it suitable for many applications. In clothing and home furnishings, nylon is an important fiber, especially in socks, stockings, lingerie, stretch fabrics, sporting goods, carpets, luggage, and materials used in upholstery of furniture. Industrial uses of nylon fiber include automobile tires, ropes, seat belts, parachutes, and substrates for coated fabrics such as artificial leather, shower and fire hoses, and disposable clothes for the health-care industry. In addition, engineering plastics made of nylon exhibited high melting points; strength, toughness, and chemical inertness, and therefore, they found immediate use as bearings, zippers, gears, automobile fan blades, and pulleys.

Tab. 1 Worldwide annual production of nylon, 1999 ($\times 10^3$ ton)

| Country | Filament | Staple | Sum |
|-------------------|----------|--------|------|
| USA, Canada | 1266 | 353 | 1619 |
| West Europe | 569 | 115 | 684 |
| China | 319 | 28 | 347 |
| Taiwan | 296 | 1 | 297 |
| Korea | 277 | 6 | 283 |
| Japan | 174 | 9 | 183 |
| East Europe | 171 | 11 | 182 |
| Rest of the world | 315 | – | 315 |
| Sum | 3387 | 523 | 3910 |

5 Biodegradation

From recent biochemical and genetic studies, it has been found that microorganisms can degrade various synthetic polyamides. Here, biodegradations of nylon, ϵ -caprolactam (initial material of nylon-6 production), and nylon oligomers (by-products of nylon manufacture) are described.

5.1

Biodegradation of Polyamides

The biodegradation of nylon-6 utilized in particular intrauterine devices (IUD) in vivo has been reported (Hudson and Crugnola, 1987). For periods of two years or longer, 67% of these devices examined had breaks in the coating of the tail string. From results using synthesized specimens of ^{14}C -labeled nylon-6,6 exposed, in vitro, to a number of enzyme solutions, nylon-6,6 was found to be unaffected by esterase, but degraded by papain, trypsin, and chymotrypsin, though the extent of degradation was small (Smith et al., 1987).

Recently, biochemical studies on the biodegradation of nylon-6,6 by a lignin-degrading fungus were reported (Deguchi et al., 1998; Nomura et al., 2001). A nylon-degrading enzyme was found in the culture medium of a white rot fungus strain. The characteristics of the purified protein such as molecular weight, absorption spectrum, and requirements for 2,6-dimethoxyphenol oxidation were found to be identical to those of manganese peroxidase. The nylon-degrading activity did not depend on exogenous H_2O_2 , but was inhibited by catalase. These features are identical to those of the reaction catalyzed by horseradish peroxidase. From the nuclear magnetic resonance (NMR) analysis of the degradation products, it was proposed that the methylene group adjacent

Tab. 2 Major companies producing synthetic polyamides

| Country | Company (ies) |
|-------------|--|
| Belgium | BASF Antwerpen N.V. (6), Beaulieu-Nylon (6), DuPont de Nemours (Belgium) (6) |
| Canada | BASF Canada (6), DuPont Canada (6-6) |
| China | China Jinlun Group (6), Guangdong Xinhui Meida Nylon (6), National Shenma Group (6-6), Liaoyang Petrochemical Fibre (6-6) |
| France | ATOFINA (6, 11, 12), Filartois (6), Kermel (Aramide), Nylstar (6-6), Rhodia Engineering Plastics S.A.(6, 6-6), Rhodia Perf. Fibres (6-6) |
| Germany | ATOFINA Deutschland GmbH (6, 6-6, 12), BASF Aktiengesellschaft (6, 6-6), Bayer AG (6, 6-6), Degussa AG (12, 6-12), DSM Deutschland GmbH (6-6), DuPont de Nemours (Deutschland) GmbH (6-6), Honeywell Polymers GmbH (6, 6-6), Leuna-Miramid GmbH (6, 6-6), Nylstar (6, 6-6), PLASTOMID Polymere GmbH (6), TWD (6-6) |
| Italia | Aquafil SpA (6), Bemberg (6), Caffaro Flexible Packaging S.p.A. (6, 6-12), Nilit (6-6), Nylstar(6, 6-6), Radici Chemical SpA (6, 6-6), Radici Fil (6), Rhodia Engineering Plastics S.r.l. (6, 6-6), Rhodia Perf. Fibre (6), Textile Produkte (6) |
| Japan | Asahi Chemical Industry Co. Ltd. (6-6), Atofina Japan (11), BASF Japan (6, 6-6), Bayer Ltd. (6, 6-6), Daicel-Degussa Ltd. (6-12, 12), Dainippon Ink and Chemicals Ltd.(6), DSM JSR Engineering Plastic Ltd. (4-6), DuPont Japan Ltd. (6-6, 6-12), EC-Showa Denko(6, 6-6, 12), Kanebo Ltd. (6), Mitsubishi Chemicals Corp. (6-6), Mitsubisi Engineering-Plastic Corp. (6, Aramide), Mitsui Chemicals Inc. (Aramide), Sumitomo Bakelite Co. Ltd. (6, 6-6, 12), Takayasu Co. Ltd. (6, 6-6), Teichu Co. Ltd. (6, 6-6), Torey Industries Inc. (6, 6-6, 6-10, 11, 12), Torey Monofilament Co. (6), Toyobo(6), Ube Industries Ltd (6, 6-6, 12), Unitika Ltd. (6-6-6), |
| Korea | Hyosung (6), Kohap (6), Kolon Ind.(6) |
| Netherlands | DSM Engineering Plastics (6, 4-6), DuPont Dow Elastomers BV (6, 6-6), Twaron Products (Aramide) |
| Spain | Dupon Iberica (Aramide), Nurel (6), Nylstar (6, 6-6), Polibasa (6, 6-6), Poliseda, S.L. (6) |
| Switzerland | EMS-CHEMIE AG (6, 6-6, 12) |
| UK | DuPont (U.K.) Ltd. (6-6, Aramide) |
| USA | ATOFINA Chemicals Inc.(11,12), BASF Corp.(6, 6-6), Bayer Corp. (6, 6-6), Beaulieu of America (6), DSM (6, 6-6, 4-6, 6-12), DuPont (6-6, 6-12, Aramide), EMS-CHEMIE (North America) Inc. (6, 6-6, 12), Honeywell (6), Nyltech North America Inc. (6, 6-6), Polymeric Resources (6, 6-6), Shaw Industries (6), Solutia Inc. (6-6), Ticona LLC (6-6), Wellman Inc. (6, 6-6) |

Types of polyamides produced by each company are shown in parentheses: 6, nylon-6; 6-6, nylon-6,6; 4-6, nylons-4,6; 6-10, nylon-6,10; 6-12, nylon-6,12; 11, nylon-11; 12, nylon-12.

to the nitrogen atom in the polymer chain was attacked by the enzyme, and subsequently the reaction proceeded auto-oxidatively (Figure 3) (Nomura et al., 2001).

5.2

Biodegradation of ϵ -Caprolactam

Owing to the polluting and toxic nature of ϵ -caprolactam, which is included as unreacted

monomer in wastewater of nylon-6 factories, its removal from waste streams is necessary. Consecutive stages of metabolisms of ϵ -caprolactam used by a sole source of carbon and nitrogen have been studied (Fukumura et al., 1982). Recently, a *Pseudomonas aeruginosa* strain, MCM B-407 was isolated from activated sludge to treat waste from a factory producing nylon-6 (Kulkarni and Kanekar, 1998). This organism was able to remove ϵ -

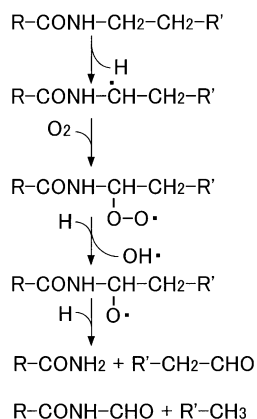


Fig. 3 Proposed mechanisms of nylon degradation by fungus peroxidase (Nomura et al., 2001).

caprolactam with simultaneous reduction in chemical oxygen demand (COD). The degradation of ϵ -caprolactam in wastewater was found to be optimal over a wide range of pH from 5.0 to 9.0, at a temperature of 30°C.

Genes responsible for the metabolism of organic compounds were sometimes coded on plasmids, which were termed as “degradative plasmid”. In *Pseudomonas*, catabolic pathways, such as those for toluene, camphor, salicylate, alkanes, naphthalene, are dependent on plasmids called TOL, CAM, SAL, OCT, and NAH plasmids, respectively (Frantz and Chakrabarty, 1986). From the conjugation experiment, it was suggested that the plasmids detected in several *Pseudomonas* strains determine the ability to utilize the ϵ -caprolactam (Boronin et al., 1984).

5.3

Biodegradation of Nylon Oligomer

Two bacterial strains, *Flavobacterium* sp. KI72 (Kinoshita et al., 1975) and *Pseudomonas* sp. NK87 (Kanagawa et al., 1989) that can degrade 6-aminohexanoate-cyclic dimer, a byproduct of the nylon-6 industry, have

been isolated. In both strains, 6-aminohexanoate-cyclic-dimer hydrolase (EI, EC 3.5.2.12) (Kinoshita et al., 1977) and 6-aminohexanoate-dimer hydrolase (EII, EC 3.5.1.46) (Kinoshita et al., 1981) are responsible for the degradation of the cyclic dimer. Here, biochemical and genetic aspects of nylon oligomer biodegradation are described.

5.3.1

Biochemistry of Nylon Oligomer

Biodegradation

Biochemical studies revealed three enzymes that are responsible for the degradation of 6-aminohexanoate oligomers, namely, 6-aminohexanoate-cyclic-dimer hydrolase (F-EI for *Flavobacterium* sp. KI72, P-EI for *Pseudomonas* sp. NK87) (Kinoshita et al., 1977; Kanagawa et al., 1989; Tsuchiya et al., 1989), 6-aminohexanoate-dimer hydrolase (F-EII for *Flavobacterium* sp. KI72, P-EII for *Pseudomonas* sp. NK87) (Kinoshita et al. 1981; Kanagawa et al., 1993), and endo-type 6-aminohexanoate oligomer hydrolase (EIII) (Negoro et al., 1992; Kakudo et al., 1993). The characteristics of the purified enzymes stated above are listed in Tables 3 and 4.

F-EI and P-EI are homodimer enzymes with a subunit molecular weight of 52,000 daltons (da). From the results of DNA sequencing of the cloned genes, as described below, the EI enzyme from F-EI and P-EI was found to be composed of 493 amino acid residues. The amino acid sequence of the P-EI enzyme was almost identical to that of the F-EI (99% homology) (Tsuchiya et al., 1989). The EI enzymes were active only toward the cyclic dimer, and not toward more than 100 kinds of natural amide bonds tested.

F-EII and P-EII were also homodimer enzymes with subunit molecular weight of 42,000 Da. In contrast to the high homology between the two EI enzymes, the EII had