surfactant science series
volume 69

ENZYMES IN DETERGENCY

edited by
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ENZYMES IN DETERGENCY

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an informa business

Reprinted 2010 by CRC Press
CRC Press
6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK

Library of Congress Cataloging-in-Publication Data

Enzymes in detergency / edited by Jan H. van Ee, Onno Misset, Erik J. Baas.
p. cm. — (Surfactant science series ; v. 69)
Includes index.
ISBN 0-8247-9995-X (alk. paper)
1. Detergents. 2. Enzymes—Industrial applications. I. Ee, Jan H. van.
II. Misset, Onno. III. Baas, Erik J. IV. Series.
TP992.5.E58 1997
668'.14—dc21

97-1912
CIP

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This book is printed on acid-free paper.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Preface

Household cleaning is a daily worldwide activity, which means that billions of people use detergents to clean their clothes, dishes, and so forth. In addition to the major ingredients, which are the surfactants and builders, enzymes are increasingly added to achieve "catalytic cleaning," thereby creating smaller fragments of the stain constituents, which in turn facilitates the chemical cleaning action of surfactants. The use of enzymes is growing constantly, not only in volume but also in types of enzyme; since the initial introduction of proteolytic enzymes some 40 years ago, present-day detergents have come to contain lipases, amylases, and cellulases. Household detergents are the most important application of industrial enzymes, representing approximately 50% of total sales.

Much progress has been made in the last decades in elucidating the structure–function relations of enzymes. Also, more has become known about the cleaning mechanisms of enzymes, and production and formulation techniques have also been considerably improved. This volume of the Surfactant Science Series contains overviews of all these different aspects as they relate to the use of enzymes in laundry and dishwasher detergents: their application, development, screening, protein engineering, manufacturing, safety, markets, and such. The combination of all these different subjects in one volume renders this book unique because no other publication of comparable broadness and depth exists.

The editors were very pleased with the overwhelmingly positive response of the major companies involved—both detergent manufacturers and enzyme suppliers—in participating in the realization of this book. Needless to say, this broad participation augments the book's value. The authors are all authorities in their respective fields. As in other volumes of this series, chapters were not required to

adhere to the same format. The authors were free to present and interpret data from their own viewpoint and experience.

During the preparation of this book, which began in the summer of 1994, major changes occurred in the world of enzyme manufacturers. The editors and authors who originally worked for Gist-Brocades moved to Genencor International in June 1995, when the latter company acquired the industrial enzymes business. Likewise, the authors originally working for Solvay became Genencor employees in July of 1996. Consequently, the book now contains many chapters that are written by Genencor authors; nevertheless, the "history" of those authors ensures that knowledge of detergent enzymes not only from Genencor International, but also from both Gist-Brocades and Solvay is represented in this work.

Finally, the editors express their deep gratitude to all the authors involved and to Joseph Stubenrauch of Marcel Dekker, Inc. for his encouragement and helpful suggestions. The support of Gist-Brocades in the initial stage of the editing process and later Genencor International, Cargill B.V., and Business Innovation Partners B.V. in permitting the editors to undertake their task is highly appreciated.

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ENZYMES IN DETERGENCY

1

Historical Overview

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I. INTRODUCTION

This chapter gives a historical overview of the development of washing habits and detergent enzyme usage from prehistoric times up to modern times. For the sake of convenience the most important dates and events are summarized in Table 1.

II. PREHISTORIC TIMES

In prehistoric times people protected themselves against cold with warm garments, which basically were made from sheep fleeces. These fleeces can be quite smelly, especially when wear is in a rather wet climate. Therefore, it was felt necessary to clean these "garments," one way or the other. This was not an easy

TABLE 1 Historical Overview of Detergent Enzymes Development

Prehistoric times	
5000 BC	Freshwater scouring in the river
	Mud trampling
	Feed mechanics
3500 BC	Soapworth, wood ashes, soda, bat
2100 BC	Soap from olive oil, wood ash
1000 BC	Fat, oil, and natron
From Romans until Röhm	
	Stale urine presoak, mud trampling
700	Soap from quicklime and fragrances
1700	Soap from fat and soda ash
1900	Soap, silicate, perborate
1928	Alkylsulfates, synthetic detergents
Modern times	
1931	Pancreatic enzymes/Burnus; Otto Röhm
1959–1965	Alcalase, Maxatase/Bio 40, Biotex; Schnyder, Kortman
1971	Termamyl, Maxamyl; Novo, Gist-Brocades
Mid-1970s	Maxatase L, Alcalase L; Gist-Brocades, Novo
1982–1985	Savinase, Maxacal; Novo, Gist-Brocades
	Opticlean; Solvay
	BLAP; Henkel
	Purafact; Genencor Int.
1986	Celluzyme; Novo
	BPN' protease; P&G–Genencor Int.
1987–1989	KACellulase; Kao
1988	Maxapem; Gist-Brocades
	Lipolase; Novo
1989	Durazym; Novo
1992	Carezym; Novo–P&G
1994–1995	Purafect OxP; Genencor Int.
	Lipomax; Gist-Brocades
	Lumafast; Genencor Int.
	Properase; Gist-Brocades–Genencor Int.

task, because fleeces contain dirt, such as fecal matter, plant remnants, dried sweat, and in particular (wool) grease, with all the adventitious filth stuck to it. Originally, primitive humans (read women) took their "clothes" to the river for freshwater scouring, seeking places with very soft water, because grease removal in hard water was much more difficult [1].

In about 5000 BC, it was discovered that woolen garments were cleaned more effectively when they had been trampled into the river mud and rinsed several times afterward to remove the mud, which carried away most grease and the dirt attached to it. Later on, this water- and clay-based–cleaning process was carried out by bare-legged women standing in a wide wooden tub, supplying with their feet the mechanics of this prototype washing machine. Around 3500 BC, it became common practice to use other "detergent" ingredients, such as soapworth, wood ashes, and soda, which gave extra-cleaning power, owing to a higher alkalinity. This resulted in the use of a special bat (Fig. 1) to beat the clothes, because the alkali would have damaged the skin of the feet [1].

From the historical Sumerian Lagash, it is known that at about 2100 BC, the Sumerians, who lived in the watershed of the Euphrates and the Tigris rivers, made soap from olive oil and wood ash. Before boiling, these two raw materials were to be mixed in fully described proportions, the recipe of which, in fact, represents the oldest record of a chemical reaction. The resultant soap was used mainly for the washing of woolen clothes. In Egypt, the most common detergent, at about 1000 BC, was a mixture of animal fat or vegetable oil with natron (which was essentially sodium carbonate found in the Wadi Natrun Desert), optionally further mixed with a kind of fuller's oil, that seems to have been what nowadays is called lanolin [1].

FIG. 1 Women washing at the river. Woodcut by Hans Franck (1526). (From CIBA Review 5:2037.)

III. FROM ROMANS UNTIL RÖHM

The Romans did not have soap. They used to presoak pieces of cloth in stale urine, which was collected systematically in large vessels placed at the corners of the streets. Subsequently, the fabrics were trampled on in a vat containing water and mud, after which several rinses were given with clean water [1]. Not until the seventh century, did the use of soap, as an important aid for cleaning clothes, grow significantly. At that time, it was discovered by the Arabs that the use of quicklime allowed the preparation of much harder soaps. This technology spread all over Europe, but specifically, in the Mediterranean area the manufacturing of notably cosmetic soap was blooming, owing to the availability of fragrant plants. It took until the late 1700s, when new technologies for soda ash manufacturing had been developed, that soap became available to people of all classes. Soap powders became very popular in the late 1800s, mainly for reasons of convenience; because new mixing methods for soda, silicate, and perborate allowed one-step washing and bleaching. The next step in the further improvement of detergents, was to find a better alternative for soap to overcome its biggest disadvantages, such as the high alkaline reaction and the sensitivity toward hard water. After a series of attempts, which led to good wetting agents with rather poor detergency properties, the first synthetic substances with excellent wetting and detergency characteristics were discovered in 1928 by the sulfation of fatty alcohols. After this discovery, low-cost quantities of these alkylsulfates rapidly became available through large-scale chemical reduction of fatty acid esters into fatty alcohols. By this route commercial volumes of syndets (synthetic detergents) could be produced (about 1930), which had better-cleaning properties than natural soap.

IV. MODERN TIMES

At the same time, in 1931 to be precise, the original idea of using enzymes was described by Dr. Otto Röhm. He patented [2] the use of pancreatic enzymes in presoak detergent compositions, to improve their ability to remove stubborn proteinaceous stains. Also in that same year, the first enzymatic detergent, named "Burnus," was launched. Despite the theoretical possibilities, the practical use of enzymes did not quite become a success because the enzymes could be made available by extraction of pancreatic glands in only limited amounts. Moreover, the functional enzymes (e.g., trypsin and chymotrypsin), with their pH optimum between 7 and 9, are not optimally suited for use in alkaline (presoak) detergents. Because of these limitations, these products were sold only moderately until the late 1940s. The first detergent containing a bacterial protease—Bio 40, produced by Schnyder in Switzerland—appeared on the market in 1959, quickly followed

by the very successful launch of Biotex in 1963, by Korman and Schulte in the Netherlands (Fig. 2). The enzymes used at that time were alkaline serine proteases, from the bacterium *Bacillus licheniformis*, market by the Danish company Novo Nordisk under the trade name Alcalase, and by the Dutch company Gist-Brocades, under the trade name Maxatase [3]. The better compatibility of these enzymes with the detergent matrices greatly spurred the development of other enzymatic detergents, and enzyme sales in the period 1965–1970 grew very fast. In 1970, this rapid growth of enzyme usage was set back dramatically (see Fig. 4), especially in the United States, by the strongly negative publicity on the dustiness of the enzyme formulations, with the concomitant development of allergies by some workers in detergent manufacturing plants. Because this situation was taken very seriously by both enzyme producers and detergent manufacturers, the problem was solved relatively quickly by the development of reduced-dust enzyme granulates (Fig. 3), that provided safe handling. Consequently, since 1971, enzyme sales have again increased steadily (Fig. 4), also because of the application of amylases, such as Maxamyl by Gist-Brocades and Termamyl by Novo Nordisk, which obtained better removal of carbohydrates, such as food stains and chocolate.

FIG. 2 Biotex in 1963. (From "100 jaar Kortman Nederland B.V.," 1987.)

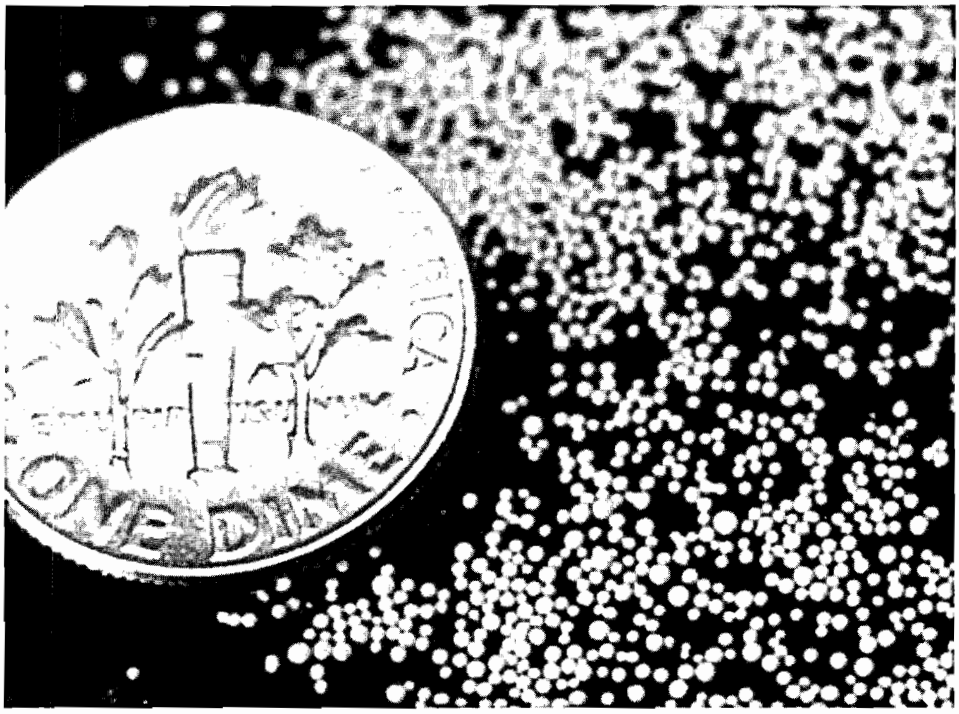

FIG. 3 Maxatase prills "for a dime."

In the mid-1970s, the share of liquid detergents became more and more important, notably in the United States and Europe, leading to the market introduction of specially developed liquid proteases, such as Maxatase LS and Alcalase L for application among others in Procter & Gamble's liquid Tide.

In the late 1970s, starting in Japan and Europe, detergents were gradually, but severely, reformulated to cope with the growth in environmental awareness, which led to the replacement of phosphates by other builders. Also, to deal with the energy crisis, the detergent manufactures were forced to develop and incorporate low-temperature bleach activators, such as TAGU (Henkel) and TAED (Lever).

Because of these developments, new proteases were needed that were more alkaline (pH 9–11), and showed a better performance at 40°–60°C. The answer came in 1982–1985 by the development and market introduction of the detergent enzymes Savinase by Novo Nordisk and Maxacal by Gist-Brocades [4] later in

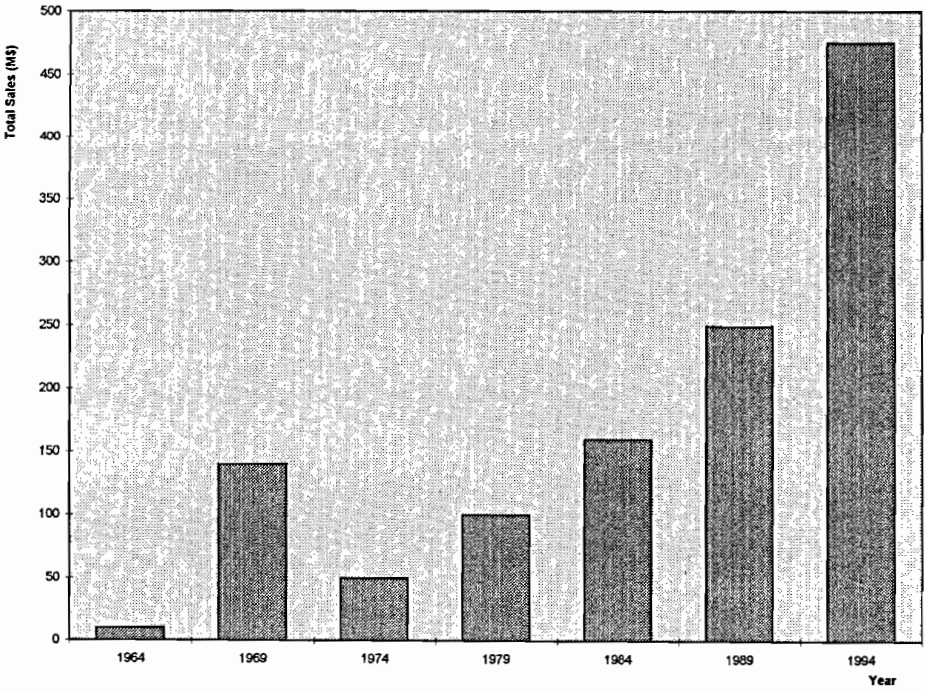

FIG. 4 Market growth of detergent enzymes.

that decade, followed by the introduction of Opticlean by Solvay, Purafect by Genencor International, and Blap by Henkel/Cognis [5].

In the late 1980s to early 1990s, several major changes in the detergent industry took place, such as nontower detergent manufacturing (dry mixing of composites), the development of concentrated heavy-duty powder detergents (initiated in Japan), the development of softening through the wash (STW) detergents, the development of concentrated/structured/nonaqueous liquid detergents, reformulation of automatic dishwasher detergents, and others. Also, a clear tendency was noted toward cleaning at lower wash temperatures, gradually shifting from 60° to 40°C, specifically in Europe.

As a consequence, the detergent enzyme industry was faced with several challenging opportunities to develop new enzymes to fill in the performance gaps that otherwise would have arisen in these new detergent formulations. Consequently, a whole series of new detergent enzymes appeared on the market, some manufactured by new entrants in the industry. For instance in 1986 Novo Nordisk introduced a cellulase, called Celluzyme, to improve textile color maintenance and to

allow softening through the wash [6,7]. About that same year Procter & Gamble (P&G) introduced a new mild alkaline protease derived from *Bacillus amyloliquefaciens*, specifically suited for boosting the performance of their liquid Tide [8]. In 1987–1989 Kao, in Japan, introduced their KAC (Kao alkaline cellulase) to boost secondary detergency at low temperatures [9,10]. To be compatible with the high concentration of (low-temperature–activated) bleach in compact detergents, new proteases were designed by new genetic technologies. As a result, Gist-Brocades in 1988, introduced their Maxapem, which was the first bleach-resistant detergent protease, obtained through protein engineering, that shows significantly improved performance, specifically after (household) storage of the bleach-containing detergents [11–13]. Novo followed about one year later with their version, called Durazym, to be followed a few years later by Purafect OxP from Genencor International.

Because of the lower wash temperature, some of the original detergent ingredients showed reduced cleaning efficacy, especially in removing fatty food stains and sebum. In 1988, Novo made the first attempt to solve this problem by the market introduction of a detergent lipase, which they called Lipolase. Although this enzyme may be used in various detergents, its performance is heavily dependent on the detergent matrix–surfactant composition, and shows benefits only after several wash cycles [14]. A few years later (1992), Novo, in close cooperation with P&G introduced a second-generation cellulase, called Carezym, with significantly improved color-brightening and softening properties.

In 1994, Gist-Brocades introduced the second-generation lipase, which is called Lipomax, and which has the capability of efficiently removing fat and sebum stains in one single-cycle wash procedure [15]. At about that same time the lipase of Genencor International, called Lumafast, was introduced.

Lately, one of the most exciting new opportunities for the use of detergent enzymes was the opening up of India, South America, and notably China, as witnessed by the vast growth in interests that, for example, P&G, Unilever, and Henkel, have taken in these geographic areas. Because of the specific wash conditions, such as low surfactant concentration, very low temperature, presoaking, and such, new enzymes were again needed to cope with these rather stringent circumstances. Therefore, Gist-Brocades in 1994, introduced a new detergent protease, called Properase, which is very efficient in low-temperature areas and in low-budget detergents [16,17].

So it is seen that, over the last decade, quite a few new types of detergent enzymes have entered the marketplace. Today it is not really an exception that modern types of heavy-duty powder detergents contain one or more different proteases for protein stain removal, an amylase for starchy food stain removal, a cellulase for color revival, softening, or secondary detergency, and a lipase for the efficient removal of fat and sebum stains.

Automatic dishwasher detergents (ADDs) have also changed rather substantially during the last decade. Traditionally, ADDs contained high concentrations of phosphate (as builder), sodium dichloroisocyanurate (as bleach), and sodium metasilicate (as bleach stabilizer), having a pH well above 11.5. Again, owing to growing environmental concern and public awareness, in the early 1980s, ADD manufacturers were forced to modify their formulations quite fundamentally. By adapting the good experience with laundry detergents, nowadays ADDs do contain activated peroxy-bleach, and builders, such as citrate or other polycarboxylic salts, and do have a moderate pH. As a consequence, the application of enzyme technology became possible, leading to modern-type ADDs that contain proteases and amylases with excellent cleaning properties [18,19].

Likewise, a growth can be noted in the application of enzymes in industrial and institutional cleaning (I&I), which sectors traditionally use rather strong chemical cleaning agents, but that are gradually now moving toward more "enzyme friendly" cleaning processes.

Therefore, it may be concluded that enzymatic cleaning is broadly applied and is expected to grow even further, specifically when new functionalities will be incorporated or even more stressful wash conditions are to be overcome everywhere in the worldwide marketplace. The only way to cope with this challenge for enzyme producers is to strive to be globally present, as exemplified by the teaming up, both from a marketing and a research and development point of view, in the recent acquisition of the detergent enzyme business of Gist-Brocades and Solvay by Genencor International.

REFERENCES

1. R. H. S. Robertson, in *Fuller's Earth a History*, Volturna Press, Hythe, Kent, 1986, pp. 1–82.
2. German Patent DE 283923 to Otto Röhm (1913).
3. C. Dambmann, P. Holm, V. Jensen, and M. H. Nielsen, How enzymes got into detergents, Dev. Ind. Microbiol. *12*:11 (1971).
4. J. H. van Ee and G. J. Verschoor, J. Com. Esp. Deterg. *17*:499 (1986).
5. C. R. Wilson, B. F. Ladin, J. R. Mielenz, S. S. M. Hom, D. Hansen, R. B. Reynolds, N. Kennedy, J. Schindler, M. Bahn, R. Schmid, M. Markgraf, and C. Paech, PCT patent appl. World Patent 9102792 (1991).
6. P. N. Christensen and V. Donalyan, Chim. Oggi *8*:41 (1990).
7. P. N. Christensen, K. Thomas, and S. Branner, Development of detergent enzymes, Proceedings of the Second World Conference on Detergents, American Oil Chemist Society, 1986, pp. 181–186.
8. M. Showell, Protein engineered detergent proteases, presented at the 82 Annual Meeting of the American Oil Chemist Society, Chicago IL, May 1991.

9. M. Murata, A new detergency mechanism based on alkaline cellulase, Proceedings of the Second World Surfactant Conference, CESIO, 1988, pp. 548–553.
10. S. Ito, S. Shikata, K. Ozaki, K. Okamoto, S. Inoue, A. Takei, Y. Ohta, and T. Satoh, Agric. Biol. Chem. *53*:1275 (1989).
11. J. H. van Ee, L. S. J. M. Mulleners, W. C. van Rijswijk, and R. A. Cuperus, J. Com. Esp. Deterg. *19*:257 (1988).
12. J. H. van Ee, J. Com. Esp. Deterg. *22*:83 (1991).
13. J. H. van Ee, Chim. Oggi *9*:31 (1991).
14. A. Aaslyng, E. Gormsen, and H. Malmos, SCI, Book of Abstracts on Recent Advances in the Detergent Industry, Cambridge, 1990, p. 59.
15. M. Crossin and H. B. M. Lenting, Inform *6*:473 (1995).
16. J. H. van Ee, J. M. van der Laan, K. P. Broekhuizen, E. J. Baas, M. Bollier, and L. J. S. M. Mulleners, J. Com. Esp. Deter. *25*:123 (1994).
17. J. H. van Ee, J. M. van der Laan, E. J. Baas, and L. J. S. M. Mulleners, Inform *5*: p. 542 (1994).
18. J. H. van Ee, W. C. van Rijswijk, and M. Bollier, Chim.-Oggi *11*:21 (1992).
19. J. H. van Ee, W. C. van Rijswijk, and M. Bollier, Proceedings of the Third World Conference on Detergents, 1993, p. 204.

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Detergent Enzymes' Market

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I. SUMMARY

The market for detergent enzymes has always had periods of rapid growth in response to the introduction of technical advances to the marketplace. Initially, rapid growth came through the introduction of protease; recent rapid growth has been driven by introductions of cellulase and lipase. From 1990 to 1995, the detergent enzyme market experienced an annual growth rate of nearly 16%; this translates into a world value of 515 million dollars today. This 515 million dollar market is expected to continue to grow at a slower rate until the next series of enzyme innovations are commercialized—then once again the market growth will escalate rapidly.

II. INTRODUCTION

A. Evolution: 1960s–1980s

Since 1961 when Bio 40 was introduced with Novo's Alcalase, detergent enzymes have proceeded on a tremendous growth track [1,2]. By 1969, at least 50% of European detergents were offering "biological attack"; the usage of which continued in these detergents at a 50% penetration rate through the 1970s and reached almost 75% in 1987. In Japan, proteolytic enzymes were introduced by Lion in 1979, and achieved a great commercial success.

After a rapid start in the United States, enzyme growth suffered a setback in 1971, when factory workers developed health problems, and detergent producers withdrew the enzymes from their products [1,3,4]. Enzyme manufacturers remedied the problem by introducing a dust-free, prilled product form, and detergent manufacturers introduced effective dust-handling systems [1,3]. Detergents with enzymes remained a minor factor in the U.S. detergent market through the 1970s and into the early 1980s, with only 5–6% of detergents containing enzymes in 1982.

The resurrection of enzymes began in the early 1980s [2] with

1. Procter & Gamble's new Era Plus liquid-containing enzymes, which used a new nonphosphate builder system, went into test market in 1982.
2. Other new product forms, concentrated powders such as Colgate–Palmolive's Fresh Start and Procter & Gamble's Ariel, containing enzymes were then offered in the United States.
3. Procter & Gamble's liquid Tide was introduced in 1985 with enzyme, and powder Tide with its 23% market share was considered a candidate for conversion to enzymes.
4. By 1986, the U.S. share of detergents using enzymes had leapt to over 40%, up from the 15–20% share held in 1984.

Although Colgate–Palmolive and Procter & Gamble embraced enzymes in the early 1980s, Lever Brothers held back its flagship brands for several years—both in the United States and the United Kingdom. Protease enzymes' next major U.S. development was full acceptance by Lever, which finally came in the late 1980s.

B. Second-Generation and New Enzymes

In the early 1980s, lipase enzymes were identified for fat splitting, and these were introduced first in detergents in Japan in 1988 and into Europe in April 1990. In 1987 in Japan, Kao had achieved great success with the first alkaline cellulase for detergents [4]. Europe saw some use of cellulase in detergents in 1990, but they remained a novelty through 1991. The use of cellulase in the United States did

not appear until 1993. Cellulase was initially proposed as a product for fabric softeners in the 1960s, but found its first success in providing antipilling and color revival in laundry detergents. In 1993, relatively high dosages of enzymes were seen in bleach-alternative liquid products. The bleach alternative with high levels of enzyme blends marked the beginning of the current period, which is noted for the popularity of enzyme "cocktails."

Another growth market has been European automatic dishwasher detergents (ADDs); beginning in 1990, many have been reformulated to contain enzymes [5]. The shift in ADD formulations was driven by the need to reduce the use of harsh sodium silicates, and this became an opportunity to address other issues. European formulators moved to abandon phosphate and chlorine in the new formulations. Europeans were able to eliminate phosphate because, unlike other regions, separate water-softening devices based on ion-exchange are included in their machines. Efforts to introduce similar formulations in the United States have been largely ineffective because the United States does not have the ion-exchange capacity in its machines. New attempts to broaden the use of enzymes in U.S. dishwashing can be expected.

In the next decade, there is likely to be a continuation of the decline in clothes wash temperatures [6]. Wash temperatures fell in Europe in the 1970s and 1980s and consumers moved away from 95°C washes. Temperatures of 40° and 60°C are now common, but further declines are expected. In the United States, efforts are underway to lead machine manufacturers to lower-temperature wash standards [7]. This could come through legislation, or through a market pull approach from utilities or other groups. With lower temperatures, reliance on enzymes, such as lipase, to remove fatty soils will increase as existing chemical systems have difficulty with these stains at lower temperatures [8]. The other types of enzymes are also likely to benefit.

Other consumer developments are expected to drive greater reliance on enzymes in detergents. With the decline in time available for housekeeping chores owing to dual-career households, expectations are rising for detergents to remove problem stains without special treatments. Furthermore, with the rise in the cost of garments, consumers want to see extended use and, hence, will expect less aggressive detergents, for instance ones operating at lower pH, or that contain "color guard" systems.

Recently, the practice of formulating with multienzyme laundry detergent packages has increased the use of amylase, an enzyme that works on starches and one that has been available for years. Use of cellulase in fabric softener products may be possible in the near future. The introduction of peroxidase enzymes, first to prevent dye transfer, and second possibly to provide bleaching, may be expected.

III. DETERGENT MARKET: 1995

The laundry detergent markets are widely varied from area to area. In all, 17.9 million tons of laundry detergent products will be consumed in 1995. Europe and North America represent half of the market, but only about 12% of the population. Powder products predominate, whereas liquids (Table 1) hold about 10% of the market. Other product forms include detergent pastes and bars.

Regionally, Western Europe is the most important market, having the highest value, [five times higher dosage, see Chap. 3], the most competition, and the most developed products. North America and Europe are well-developed markets, characterized by low growth, equivalent to that of population growth. In the other regions, Eastern Europe holds the largest portion, representing over half of the total for the area (Fig. 1).

Other regions represent the important areas where growth is based on increased per capita use and the substitution of soap. Laundry soap represents nearly a 5 million ton market worldwide and is important still in Asia. Over the next 15 years, the substitution of soaps will provide an important engine for growth to detergent and enzyme makers.

Enzymes are also found in other detergent products, ranging from automatic dishwasher detergents to dry bleaches, stain removers, and boosters. Autodish products are found largely in Europe and North America, where together they are a 700,000 ton market. Dry bleaches are primarily found in North America and represent an annual market of over 100,000 tons.

IV. DETERGENT ENZYME MARKETS AND PRODUCERS

Novo Nordisk developed the first alkaline-stable enzyme, Alcalase, in 1958. It was developed for meat and fish packers, and since 1961–1962, when it was first used in laundry applications, Novo has held the leading market share [1,9]. Today Novo holds over 50% of the 515 million dollar detergent market (Fig. 2). Other

TABLE 1 World Laundry Detergents: 1995 (million tons)

Heavy-duty powders	6,500
Heavy-duty liquid	1,700
Others and unspecified	9,700
Total	17,900

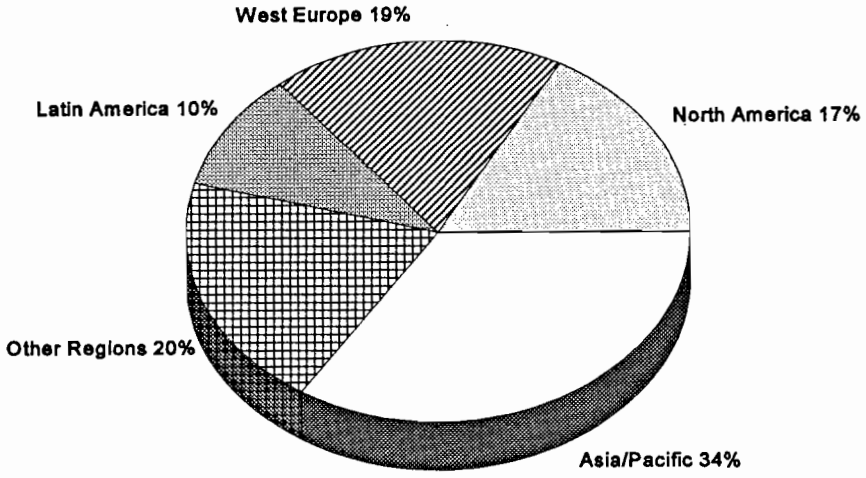

FIG. 1 Regional laundry detergent markets (1995) on a volume basis: Total = 17.9 million tons.

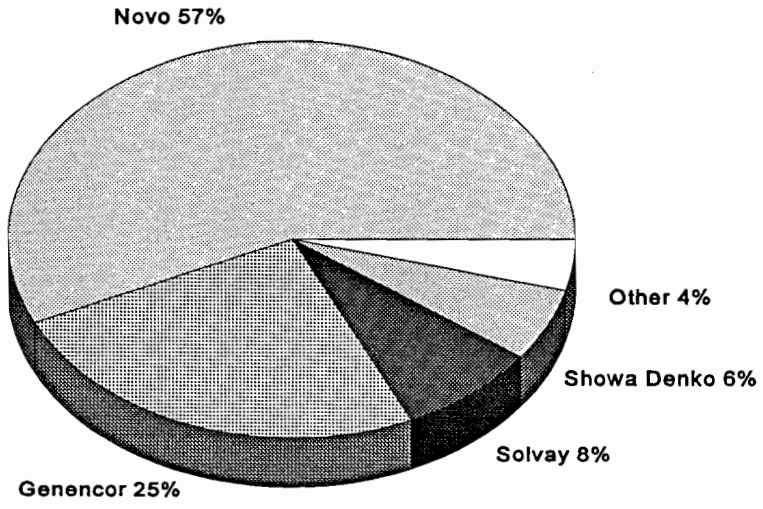

FIG. 2 Detergent enzyme merchant producer shares: 1995. (From Colin A. Houston & Associates estimates.)

producers include Genencor International, Solvay, and Showa Denko. There is captive protease production by Henkel and Kao. Kao also makes and sells an alkaline cellulase.

Although Genencor International is a modern, technology-driven company, it has a long history in enzymes, with roots tracing back to Rohm and Haas Enzymes, which was sold to Corning Glass in 1981. In 1982, Genentech and Corning created a new company, Genencor. In 1984, A. E. Staley made a substantial investment in Genencor to become a partner with Corning and Genentech. Eastman bought Staley's share and Genencor became part of Eastman Kodak. In 1990, Cultor, the former Finn Sugar, joined Eastman to form Genencor International. In 1995, Genencor International purchased portions of Gist-Brocades N.V.'s industrial enzyme business, including the detergent enzyme sector. Gist-Brocades is a classical company driven by fermentation, similar to Novo Nordisk. In December 1995, Genencor International announced that it had reached an agreement to purchase the enzyme business of Solvay [10].

There were earlier consolidations within the industry. Solvay purchased Miles Enzymes in 1990 and added it to its Kali Chemie enzyme business in Europe.

Another interesting feature of this market is the captive positions held by Henkel and Kao. Henkel produces and uses its enzymes primarily in the European region, but does some research in the United States. Kao has developed materials captively and is now looking for merchant sales opportunities. Kao was the first to succeed with an alkaline cellulase and now offers these products to other detergent producers.

Enzyme research is difficult and expensive. Close cooperation between customer and supplier is often required. At times Novo has worked with Unilever to develop protease, lipase, and cellulase products for detergents. A cellulase was developed years later in another joint effort, this time between Novo and Procter & Gamble (P&G). Today Novo's arrangement with P&G on cellulases with exclusive rights to Novo's Carezyme package is resulting in significant benefits. Between February 1995 when P&G introduced this technology, and October 1995, the Tide brand gained 3 percentage points to reach a 30% share of U.S. laundry detergent sales. Increasingly, producers are finding the development of new enzymes to be risky and costly; therefore, they have increased the success potential of projects through joint development agreements. The need to differentiate laundry products remains high and leaves detergent producers very willing to support research for one of the leading formulating technologies.

Today, production facilities are sited around the world, Novo has four locations and Genencor has four. Solvay produces in Germany, the United States and Argentina. Novo, Kao, and Showa Denko are in Japan, and Henkel is in Austria. Table 2 lists the production locations of the producers.

TABLE 2 Enzyme Production Facilities

Producer	Locations	Processes
Novo Nordisk	Kalundborg, Denmark	Fermentation, granulation
	Granklinton, North Carolina	Fermentation, granulation
	Hokkaido, Japan	Fermentation
	Aravcaria, Brazil	Fermentation, granulation
Genencor International	Cedar Rapids, Iowa	Fermentation, granulation
	Brugge, Belgium	Fermentation, granulation
	Hanko, Finland	Fermentation, granulation
	Jamsankoshi, Finland	Fermentation, granulation
Solvay	Elkhardt, Indiana	Fermentation
	Arroyito, Argentina	Fermentation
	Nienburg, Germany	Fermentation, granulation
Showa Denko	Ohito, Japan	Fermentation, granulation
Biozym BV (Henkel/Sandoz)	Kundl, Austria	Fermentation, granulation
Kao	Wakayama, Japan	Fermentation, granulation

A. Products

Each of the producers tends to have a broad product line and offers a range of strengths for each type of enzyme. Other variations include products targeting different operating conditions. For instance, some products operate well at a pH of 11, whereas others are designed to operate better at pH 9. Additional features include improved bleach or color stability [11]. Coating systems for liquid products are another point of differentiation. The strength of the enzymes is measured by proprietary methods that prevent direct comparison of enzymes between producers. Table 3 indicates the trade names offered by the producers by enzyme type.

B. Market Size

In the 1960s, enzymes experienced phenomenal growth and, in fact, were the performance growth engine to the detergent industry that optical brighteners had been in the 1950s [2]. The 1970s were a disappointment in the United States, and European enzymes held a steady 50% of detergents through the decade. The 1980s saw strong growth in the United States and Japan and from the introduction of new types; cellulase and lipase. European use of enzymes reached 75% of detergents during the 1980s and is at 95% today [9]. In another development, the encapsulation of enzymes opened up the liquid detergent market as an important new outlet. Cellulase and lipase enzymes did not really affect the Western

TABLE 3 Enzyme Product Names by Type and Supplier: 1995

	Protease				Amylase			
Producer	Alkaline	High alkaline	Bleach-stable	Cold water	Conventional	Bleach-stable	Lipase	Cellulase
Merchant market								
Novo Nordisk	Alcalase	Savinase	Durazym	—	Termamyl	Duramyl	Lipolase	Carezym
	—	Esperase	—	—	—	—	Lipolase ultra	Celluzyme
Genencor	Maxatase	Maxacal	Maxapem	Properase	Maxamyl	Purafect OxAm	Lipomax	—
International	—	Purafect	Purafect OxP	—	—	—	Lumafast	—
Solvay	Optimase	Opticlean	Opticlean+	—	Amylase MT	—	—	—
Showa Denko	—	Kazusase	—	—	—	—	—	—
Captive market								
Kao	—	KAP	—	—	KAA	—	—	KAC
Henkel	Biozym	Blap	Blap+	—	—	—	—	—

markets until the 1990s, when they provided an important growth driver [8,12,13]. Today, enzymes are the foremost performance tool available to formulators and are the basis of strategic initiatives by the detergent producers.

World use of detergent enzymes is reported to have reached over 250 million dollars in 1990 [12]. Today, the market is 515 million dollars, a growth of nearly 16% per year. This unusually high growth is due to the addition of the autodish market and to the new functionalities provided by lipase and cellulase finding use in Western markets. Such growth is unlikely to be repeated in the near future.

Geographically, the use of enzymes is shown in Figure 3. There is a high level of penetration of enzyme detergents in most areas, including Europe, Japan, and North and South America. Penetration in the United States is 75%, compared with 95% for Europe and Japan. The high value in Europe is mainly due to high levels of detergent use and high enzyme concentrations (see Chap. 3).

The mainstay of the market has been the protease types. In the United States, these products have been evolving towards "pH-neutral" types. In Europe, the pH of systems remains relatively high. Protease prices have been declining, but with increased usage, the total market value has held steady in most areas [9]. Proteases now offer very good performance value that has been seized on in the introduction of "bleach-alternative" formulations.

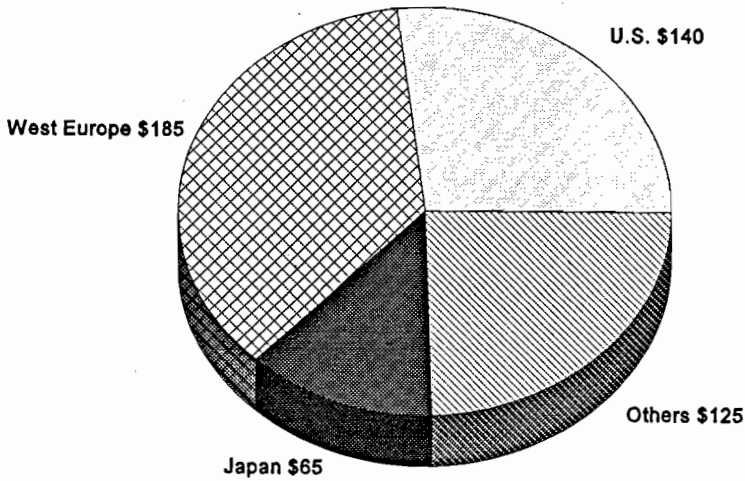

FIG. 3 Regional detergent enzyme market values: 1995, in millions of dollars.

The value of protease sales worldwide is in the region of 320 million dollars. Lowest concentration proteases sell for \$1.60–\$2.00/lb. Typically, pricing depends on

Concentration
Form
Market location
Exchange rates
Customer sizes
Level of competition

The determination of the lipase and cellulase markets size is awkward because of proprietary arrangements stemming from enzyme producers and consumers undertaking joint development efforts. Lipases have grown rapidly since 1990 [8,13] to reach an estimated 55 million dollars today. Cellulase enzymes, in contrast with lipase, have grown more rapidly since 1993 and are estimated to have reached 110 million dollars in 1995. Lipases sell for over \$4.00/lb and the market is dominated by Novo. Gist-Brocades/Genencor International has introduced a new, more active lipase (Lipomax) product that has generated great interest.

Table 4 breaks out regional market segments by type of enzyme. It is clear from the table that enzymes in detergents have become an important market. Given the speed of growth by the new materials—lipase and cellulase—considerable growth potential remains. Cellulase and lipase growth should continue to drive the market for several more years.

V. CONCLUSION

Today, enzymes have gained wide approval and are used in detergents throughout the world. Healthy growth is forecast for enzymes, and new developments in

TABLE 4 Detergent Enzyme Markets by Type and Region: 1995 (million U.S. dollars)

	Total	Protease	Lipase	Cellulase	Amylase
United States	140	80	10	40	10
Western Europe	185	100	20	45	20
Japan	65	30	15	20	—
Others	125	110	10	5	—
Total	515	320	55	110	30

Source: Colin A. Houston & Associates estimates.

enzymes are anticipated to continue to drive periods of rapid growth as detergent producers rely more heavily on the remarkable effectiveness of enzymes to release stains and certain soils under increasingly difficult wash conditions. The development of new enzymes will be well received by consumers as they continue to favor easy-care developments, which allow increased time for friends and family.

REFERENCES

1. P. Brodeur, in *Asbestos and Enzymes*, Ballantine Books, New York, 1971.
2. L. H. Dalgaard, et al., Dishwashing—a new area for enzymes. Inform 2:37 (1991).
3. P. Layman, Promising new markets emerging for commercial enzymes. Chem. Eng. News Sept. 24:17 (1990).
4. New! improved! no kidding! Forbes, Mar. 7:154 (1988).
5. C. A. Starace, Detergent enzymes: developments during the last decade. J. Am. Oil Chem. Feb:165 (1981).
6. C. A. Starace, Detergent enzymes: Past, present and future. HAPPI, Mar:49 (1983).
7. Genencor to buy solvay enzyme business. Chem. Market. Rep. Dec 4:5 (1995).
8. U. Hotz, Verwendung von Cellulasen in Waschmitteln. Tenside *30*:388 (1993).
9. D. Kochavi, Enzymes for household detergents. Inform, Sept:990 (1993).
10. E. D'Amico, Technological advances help market penetration. Chem. Week, Oct. 25:34 (1995).
11. Gist-Brocades opens new enzyme plant. Inform Feb:200 (1995).
12. M. Ward, Expanding role of enzyme culture. Eur. Chem. News Feb. 20:24 (1995).
13. J. DiCorpo, Global visions. Chem. Market. Rep. Jan. 16:18 (1995).

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Enzymes: Their Applications and Biochemical Characterization

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I. ENZYME BIOCHEMISTRY

A. Enzymes Are Proteins

Proteins are one of the most versatile and diverse group of biopolymers found in nature. Composed from a limited set of building blocks—the 20 amino acids—a wide variety of proteins can be made. Proteins have numerous functions within living organisms, ranging from maintenance of cell structure, to nutrient transport and communication, to control of the biochemical processes that are essential for life.

Enzymes are a class of proteins that function as biocatalysts. They are involved in the formation and degradation of all biological substances. The biocatalytic process will be described in more detail in the sections that follow.

The basic structure of all proteins, including enzymes, is the peptide chain, which is a liner array of amino acids. The amino acids are linked together by a peptide bond, coupling the carboxylic acid (COOH) and amino (NH_2) residues between two peptides. The peptide bond (-C(O)-NH-) and its subsequent protease hydrolysis products are depicted in Fig. 1.

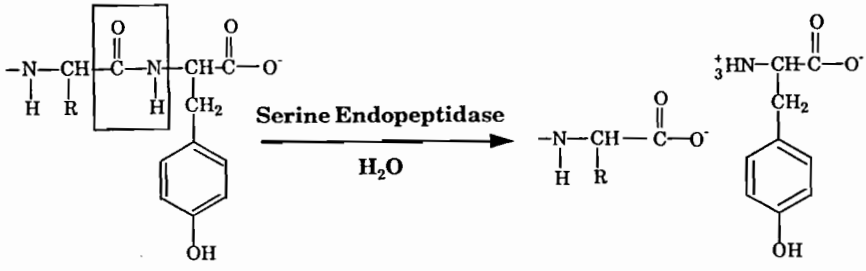

FIG. 1 Peptide bond and protease hydrolysis products. (Courtesy of Dr. Raj Lad, Genencor International, Inc., Palo Alto, CA.)

Each amino acid has specific chemical characteristics, determined by its appended side chain. Protein characteristics are determined not only by the amino acid side chains, but also by the three-dimensional structure of the polypeptide chain. For a protein to fulfill its biological function, it is essential that it has a specific, well-defined structure. Any disturbances in this structure may result in denaturation, which can lead to reduced activity.

Microbial enzymes vary in length from 100 to 500 amino acids, and typically have a molecular weight ranging from 25,000 to 50,000 Da. The active site of the enzyme is a specific three-dimensional area, within the folded enzyme, involved in substrate binding and the biocatalytic process. In certain cellulases, there is another distinct region involved specifically in substrate binding, which is referred to as the binding domain.

Enzymes are characterized by the biochemical reactions they catalyze (e.g., proteases catalyze the hydrolysis of the peptide bonds in proteins and lipases catalyze the hydrolysis of ester bonds in triglycerides and fats). Table 1 depicts these enzyme classifications [1] as specified by the International Union of Biochemistry (IUB).

B. Enzyme–Substrate Interaction: Mechanism and Kinetics

Enzymes are biocatalysts that facilitate biochemical transformations. They allow unfavorable biochemical reactions to proceed more efficiently. Enzymes carry out highly specific biochemical transformations, yet remain unchanged during

TABLE 1 Enzyme Classification

IUB classification	Mechanism of action
Oxidoreductases	Catalyze oxidation/reduction reactions (e.g., glucose oxidase)
Transferases	Catalyze transfer of a functional group between molecules
Hydrolases	Catalyze the addition of water across a bond (e.g., protease, lipase, α-amylase, and cellulase)
Lyases	Catalyze the addition of a functional group to a double bond or generate double bonds (e.g., pectin lyase [transeliminase])
Isomerases	Catalyze the isomerization or rearrangement of a molecule (e.g., glucose isomerase)
Ligases (synthetases)	Catalyze the breaking or formation of two molecules concomitant with cleavage of a nucleoside triphosphate (e.g., glutamine synthetase)

Source: Ref. 1.

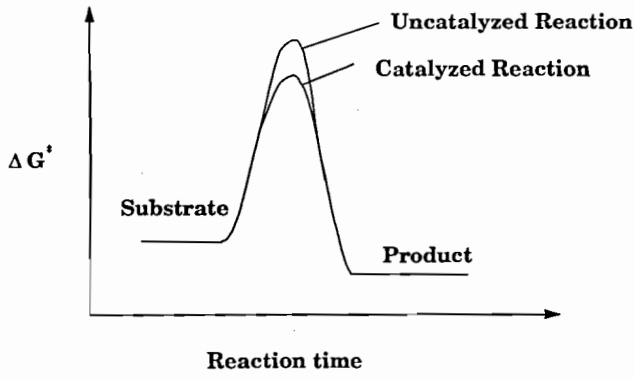

FIG. 2 Thermodynamic reaction profile. (From Ref. 1.)

this process. Enzymes increase the rate of biochemical conversion of substrate [S] to product [P] by decreasing the free energy of activation barrier ($\Delta G^{\ddagger}$); Fig. 2). Typically, enzymes can increase the rate of reaction 1-millionfold or more. In the absence of enzymes, few biochemical reactions would proceed at all.

The molecular basis of catalysis is dependent on the stabilization of the enzyme–substrate complex [ES] formed at the enzyme's active site during the catalytic reaction (Figs. 2 and 3). Figure 4 depicts the tetrahedral intermediate formed by the enzyme–substrate interaction.

The [ES] complex is stabilized both electrostatically and through van der Waals attractive forces attributable to the amino acid side chains present at the enzyme's active site. The formation of the enzyme–substrate complex helps overcome the thermodynamic barrier depicted in Fig. 2.

1. Protease–Hydrolase Reaction Mechanism

The overall hydrolysis reaction for a protein by a serine endopeptidase is depicted in Figure 1. The serine endopeptidase contains a catalytic triad of amino acids at the active site: an aspartyl residue containing a β-COO^-; a histidine containing the imidazole group; and a serine residue with a β-OH as a functional group. The serine hydroxyl group functions as a potential nucleophile, whereas both the

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_{cat}} P$$

FIG. 3 Simple enzyme–substrate reaction mode. (From Ref. 1.)