Enzyme Wave

Volume 10
2007

AMANO

Chubu News* — Ise Jingu

* The word "Chubu" translates literally as the "central part" (of Japan).
The Nagoya Region, where the headquarters of Amano Enzyme Inc. is based, is located in the central part of Japan.

Ise Jingu holds its own unique ceremony called Shikinen Sengu, which has a long tradition of about 1,300 years. Although there was a period of interruption (Warring States Period) in the long history, the ceremony has been repeated every 20 years. The next, to be held in 2013, will be the 62nd event.
The word "Sengu" refers to the ceremony of rebuilding the shrine and asking the God, Omikami, to move into the new shrine, while the word "Shikinen" translates as "the prescribed year". The Ise Jingu consists of two shrines: Naiku (Inner Shrine) and Geku (Outer Shrine). Each of the Naiku and Geku has two separate lots in the east and the west that occupy the same area. Each of the shrines is rebuilt on one of the two lots and is relocated from one lot to the other every 20 years. At the same time, the sacred apparel and treasures are also renewed.
There is no definite answer as to why they are rebuilt every 20 years. However, there are many different reasons used to explain it. For example, 20 years can be seen as an important milestone in life. In addition, 20 years is regarded as a reasonable length of time for skills to be handed down to the next generation.
Preventing the dilapidation of the shrine buildings is not the only reason that the Jingu has been carrying on in the Shikinen Sengu ceremony. The Jingu opted for a style of architecture called "yui-itsu shinmei zukuri", and has maintained its original style by rebuilding the shrines every 20 years, to remain new and yet the same forever. This is how the Jingu has always celebrated the Gods in the same way as it did long, long ago, through different times.
The tradition of repeating the same procedure every 20 years not only has helped traditional Japanese shrine carpenters, artists and craftsmen to pass on their skills to younger generations, but has also contributed to developing a culture that supports the lives and society of Japanese people.
The Sengu ceremony involves more than 30 festivals and events. Although the most climactic event, Sengyo (transfer ceremony of the symbol of Amaterasu Omikami from the old shrine to the new shrine), is scheduled for 2013, preparations towards it have been progressing steadily since as early as 2005.

Images provided by Jingu-shicho

Topics — Investment in IGM (Mexico) and the establishment of a subsidiary in China

Investment in IGM (Mexico)

In late December of 2006, we acquired full ownership of the stock of IGM (Industrial Goei de Mexico S.A. de C.V.) and renamed the company Amano Enzyme de Mexico S.A. de C.V. (AEM).
Since its foundation in 1976, IGM has been engaged in the manufacture of animal organ extracts for use in pharmaceuticals, health food, etc., mainly from pig and cow organs produced in Mexico. We will seek to expand the range of animal-derived products, in the hopes of creating good synergy with our enzyme business. The company is located in Irapuato City, 330 km northwest of the Mexican capital, Mexico City.

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The establishment of a subsidiary in China

We set up a representative office in China in December 2003 and have been conducting a rigorous marketing effort in the Chinese market, resulting in the establishment of a subsidiary in China.
Compared to the time when our representative office was set up, enzyme use now attracts much more attention in China today, as the result of the shift in the production of pharmaceutical and pesticide intermediates to China, as well as processed foods becoming increasingly popular as the economy grows.
It is predicted that the Chinese enzyme market will further expand in the future, driven by steady economic growth. Given that the production of pharmaceutical and pesticide intermediates continues to be based in China, the enzyme market for bioconversion in China is expected to grow as well. Moreover, continued growth is also predicted in enzymes used for liquid seasonings, yeast extracts and fuel alcohol.

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Introduction

In recent years, the number of diabetic patients has continued to grow. In Japan, there are approximately two million diabetic patients, and the number of candidates for diabetes is said to reach as many as 16 million. Throughout the world, it is estimated that there are 200 million people who have diabetes.
Diabetic patients, especially those who are insulin dependent, need to monitor and control their own blood sugar on a regular basis. Treatments including medication, diet, and exercise therapy are chosen based upon the amount of glucose measured in blood.
In recent years, easy-to-use self-monitoring blood glucose meters have become available for diabetic patients to monitor their own blood sugar more accurately in real time. Physicians can provide appropriate treatment thanks to measurements using this device and thus can prevent the progression of the disease and improve quality of life for the patients.
Self-monitoring blood glucose meters are used in Japan and throughout the world. In order to conduct the measurements, a small drop of blood is taken by poking the patient's finger-tip with a lancet device and the blood sample is placed on a test strip. Subsequently, blood glucose and the enzymes contained in the test strip will react to each other. The glucose level can be determined by measuring the electric current or the color generated by the enzyme reaction.
The market for products related to blood glucose monitoring is enormous for meters, disposable lancet devices, and enzyme-containing test strips. The scale of the market as a whole is said to be around 42 billion yen in Japan and around 550 billion yen throughout the world. Of these numbers, disposable lancet devices and enzyme-containing test strips constitute more than 90 percent. The market for enzymes contained in the test strips is naturally also large and therefore blood glucose monitoring is an attractive business field for enzyme manufacturers as well.

The principle of glucose measuring using a self-monitoring blood glucose meter and the problems of the enzymes used in test strips today

Major enzymes used in the test strips are glucose oxidase and PQQ-dependent glucose dehydrogenase. To explain the principle of measuring glucose, a description of the method for measuring electric current generated by the enzyme reaction is detailed as follows: After the reaction of glucose and an enzyme in the presence of a mediator, coenzymes (flavin adenine dinucleotide [FAD] and pyrroloquinoline quinone [PQQ]) are reduced and subsequently the mediator is also reduced. Then voltage is applied to maintain a constant potential level in order to electrolyze the reduced mediator. The current value decreases little by little until it stops deceasing and becomes stable. Because the current value correlates with the concentration of reduced mediator, that is, the glucose concentration, the glucose concentration can be determined by measuring the electrical current.
However, such enzymes exhibit some problems when used for glucose monitoring. Besides the mediator, glucose oxidase uses oxygen as another electron acceptor. Thus, if the blood oxygen level is high, measurements of blood glucose may appear lower than the true value. In order to avoid this problem, test strips using PQQ-dependent glucose dehydrogenase were developed.
The blood oxygen level does not affect these test strips because the enzyme does not use oxygen as an electron acceptor. The enzyme is also superior in its reaction speed to glucose oxidase.
The problem is, however, PQQ-dependent glucose dehydrogenase is not substrate specific. Other than glucose, it reacts with maltose, galactose, maltotriose, etc. This enzyme profile has lead to the report of a patient who developed hypoglycemia after using a self-monitoring blood glucose meter. For this patient, the glucose level measured was higher than its true value because of maltose that was contained in the patient's blood. The patient received insulin on the basis of the measurement and developed hypoglycemia. Another problem of PQQ-dependent glucose dehydrogenase is its instability compared to glucose oxidase.

The discovery of FAD-dependent glucose dehydrogenase

Taking into account the problems above, we screened enzyme-producing microorganisms from sources including soils from different areas, and isolated a microorganism that can produce an enzyme that satisfies our purpose. After examining conditions for enzyme production, we purified the enzyme and obtained an electrophoretically homogeneous sample.
This enzyme, FAD-dependent glucose dehydrogenase, contained sugar and flavin adenine dinucleotide (FAD) and had a molecular weight of approximately 400 kDa. We also evaluated the substrate specificity of this enzyme in comparison with glucose oxidase and PQQ-dependent glucose dehydrogenase. As a result, we found that FAD-dependent glucose dehydrogenase specifically reacted with glucose as it would with glucose oxidase, as shown in Table 1.
In addition, a comparative evaluation between the reaction of this enzyme whereby oxygen is used as an electron acceptor and the reaction using mediator as an electron acceptor showed almost no indication of the former reaction. It is suggested that the measurement of glucose using a test strip that incorporates FAD-dependent glucose dehydrogenase would enable an accurate determination of glucose levels without any influence from the dissolved oxygen concentration in the sample.
Furthermore, it revealed that the enzyme kept stable for long periods in various environments, which is a profile that is essential for enzymes to be used in test strips.

Conclusion

As mentioned above, FAD-dependent glucose dehydrogenase, as we discovered, is an enzyme that can use to solve conventional problems attributable to enzymes. Test strips using this enzyme are expected to determine blood glucose more accurately, or to be more specific, measurements of glucose would not be affected by the dissolved oxygen concentration in the sample (an improvement over the problems exhibited by glucose oxidase), and the measurements are expected to be specific to glucose (an improvement over the problems exhibited by PQQ dependent glucose dehydrogenase). In addition, its favorable stability promises the practicability of FAD-dependent glucose dehydrogenase (an improvement over the problems exhibited by PQQ dependent glucose dehydrogenase).
In the future, it is expected that the number of diabetic patients will grow increasingly throughout the world. Easy-to-use self-monitoring blood glucose meters must be developed further to eliminate pain during blood drawing or even actually removing the necessity for blood drawing. Against this background, the application of FAD-dependent glucose dehydrogenase to next generation self-monitoring blood glucose meters is greatly anticipated.

(Table 1) Substrate Specificity of FAD-dependent Glucose Dehydrogenase, Glucose Oxidase and PQQ-dependent Glucose Dehydrogenase

Substrate	FAD-dependent Glucose Dehydrogenase	Glucose Oxidase (Relative activity %)	PQQ-dependent Glucose Dehydrogenase
Glucose	100	100	100
Maltose	0	0	98
Galactose	0	0	15
Malttriose	0	0	63
Malthexaose	0	0	22

In recent years, the importance of "white biotechnology" is being focused on especially in U.S. and European countries.

There are mainly three applications of biotechnology processes: medical (red biotechnology), agricultural (green biotechnology), and industrial processes. Biotechnology applied to industrial processes is called "white biotechnology", which will have a great impact on future industrial production as well as enormous potential for creating a sustainable environment and society. White biotechnology is mainly based on biocatalysis and fermentation technology.

Detailed case studies conducted by leading companies operating in white biotechnology and a market analysis by the global consultancy firm McKinsey&Company confirmed that the social(People), environment(Planet) and economic(Profit) benefits(The Triple-P benefits) of white biotechnology go hand in hand. It was also estimated that 10 to 20% of all chemicals would be produced using biotechniques by 2010. Today the level is approximately 5%, and is presumed to develop at a rate of 11 to 22 billion Euro/year. In the field of fine chemicals, biotechniques are expected to be responsible for producing up to 60% of the products by 2010.

Conventionally, polymers have been produced from exhaustible fossil resources such as petroleum and natural gas. Meanwhile, biotechniques have developed polymers using renewable resources such as sugar and corn and some biopolymers have been marketed already. One example is NatureWorks® developed by Cargill Dow LLC (U.S.) using corn as the raw material. NatureWorks® is used for a variety of purposes including clothing and packaging.

White biotechnology also contributes to energy production using biomass as a renewable resource. Manufacturers currently produce ethanol from corn starch, potato starch, or cane sugar as a substitute for gasoline. Henry Ford planned to use ethanol as the primary fuel for his initial Model-T Ford. Currently in Brazil, pure or 20% ethanol derived from cane sugar is marketed as a substitute for gasoline. In the United States, 10% ethanol (E10 fuel) constitutes 30% of automotive fuels. Approximately 18% of the corn produced in U.S. is used for ethanol production. In Europe, the proportion of biofuels is projected up to 5.75% for use in automobiles. The current level is 0.3%. In order to achieve the targeted percentage, it is necessary to produce or import 9.3 million tons of biofuels per year by 2010.
It is one of the key strategic challenges of the 21st Century to create a sustainable environment and society using new developments in biotechniques. In 2005, the OECD launched a two-year project named "The Bioeconomy to 2030".
The bioeconomy is a new concept that encompasses many economic activities- each of which benefits from new discoveries, related products and services arising out of the biosciences.

European countries possess high-level accumulations of white biotechnology. They produce 70% of the enzymes in the world. In addition, the governments as well as the people are quite aware of the necessity to build a sustainable society. In the United States, they place the development of white biotechnology as a key strategy and invest in research and development nearly 10 times more than European countries do. Representatives from different governmental bodies, industry, agriculture, and academia worked together on a project called "Vision 2020."

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May.2007