

Keeping “Eyes” on the

Human bodies are made of proteins. Grasping their form should give us an understanding of the structure of our bodies. The mass spectrometer is an instrument that lets us see how proteins work inside the body. Shimadzu Corporation pro-Shimadzu developed a new method that dramatically simplifies protein analysis. The discovery of idiosyncratic proteins during disease is expected to revolutionize drug development and medical treat-

Proteomics — a Business of the Future

Dr. Osamu Nishimura is a pharmaceutical specialist who participated in the development of many drugs at Takeda Chemical Industries, Ltd. He has a unique background among the mechanical, electrical and applied physics spe-



Osamu Nishimura

Senior Corporate Officer, Shimadzu Corporation
Deputy General Manager, Analytical & Measuring Instruments Division
General Manager, Life Science Laboratory
Manager, Proteome Analysis Center

Joined Takeda Chemical Industries, Ltd. after obtaining a master's degree from Graduate School of Pharmaceutical Sciences, Kyoto University.

Successfully became General Manager of the Takeda Biotechnology Research Laboratory and Pharmaceutical Discovery Research Division. Joined Shimadzu Corporation in 2001. Doctorate in Pharmacy
Visiting Professor at Osaka University

cialists at Shimadzu Corporation.

Nishimura moved to Shimadzu in July 2001. Former Takeda president Masahiko Fujino (currently Corporate Advisor) had been Nishimura's immediate superior from the time he entered the company. There was a reason why Fujino let his favorite disciple, who held the important position of General Manager of the Pharmaceutical Discovery Research Division, go to Shimadzu.

As the analysis of the human genome progressed, the focus of pharmaceutical development shifted to genome-based drug discovery. This is a method to develop the most effective drugs to combat a disease through investigations into the idiosyncratic actions of genes during the disease. Takeda was close to completing a system for research on drug discovery based on genomic information.

However, Nishimura, as a scientist, wasn't entirely satisfied. "Genes are the blueprints for proteins, and the body is essentially composed of proteins. These proteins change ceaselessly moment by moment due to the effects of hormones, water, and other proteins in the body. I came to think that we couldn't make effective drugs without investigating the actions of such proteins."

The future lifeline of pharmaceutical development is simply the careful examination and study of protein actions. Fujino certainly believed this as well. So they sought a setting where they could intensively conduct their investigations into proteins.

At the time, Shimadzu was establishing itself in the promising business field of protein analysis, centered on its "soft laser desorption/ionization methods."

Shimadzu Corporation had many excellent mechanical, electrical and applied physics specialists, but it lacked biologists and chemists. Top management realized that people with a comprehensive knowledge across the field were essential to seriously tackle the life sciences, and began headhunting. Thus Nishimura was singled out as a person who could effectively bridge the expertise of the two companies.

"After being appointed, my first task was to establish the Life Science Laboratory in Shimadzu. Due to the lack of experts in biology and chemistry at the company, first I had to gather personnel from a variety of places," recalls Nishimura.

Tagging Amino Acids

The Life Science Laboratory, which initially started with just ten staff members, has achieved a remarkable result. It developed a new analytical method to discover proteins in blood or tissue that are generated during an illness.

Trace amounts of proteins not normally found are generated when an illness occurs, creating a different protein balance from the healthy state. The compilation of data on diseased and healthy states will possibly permit the accurate diagnosis of the name and severity of the illness in the future simply by investigating the amounts of protein present.

Proteome analysis, as a field of life science, is currently generating the most interest around the world. This analysis method might form the launch pad for developing medicines based on protein information.

Nishimura is the central figure in this development.

Nishimura focused on the amino acid tryptophan for a method of efficient proteome analysis.

Tryptophan is an amino acid that plays an important role in proteins. It occurs in almost all of the tens of thousands of proteins but the amount contained in one individual protein is among the lowest of all amino acids.

Nishimura's idea was to "tag" tryptophan. He

ground up cells taken from the body and added tags to the tryptophan in the sample. He then used a mass spectrometer to list the proteins in the sample sequentially from lightest to heaviest.

The Eye That Studies the Body

This method was applied to the cells of healthy and diseased subjects. The tryptophan of



Stable Isotope Identification Kit for Proteome Expression Analysis

Path to Proteomics

and the overall mechanisms of life, including memories and thoughts. duces the premier mass spectrometers and protein analysis methods. In July last year, ments. Osamu Nishimura and Koichi Tanaka were the key to this development.

healthy people was tagged, for example, “green”, and the tryptophan of diseased people was tagged “red”. They were then mixed together. This resulted in a matching number of “green” and “red” tags for most proteins, but some non-matching tags were also discovered. These proteins change in quantity idiosyncratically during an illness.

A database of diseases and the idiosyncratic increase or reduction in proteins correlating to each disease can be created from data accumulated by repeatedly applying this method. This should allow disease identification from a simple blood test.

The tags used in practice are carbon isotopes. Tags incorporating the carbon isotopes ^{12}C and ^{13}C , which have different masses, were attached to the tryptophan.

Nishimura’s extensive knowledge of biochemistry was a major factor in focusing on tryptophan. Although significant research is being conducted around the world, few successes have been achieved so far. Most of these attempts use the amino acid cysteine as the target.

Nishimura explains, “After long years of studying the human body, I had a gut feeling that we should choose tryptophan to investigate the amounts of protein.”

“The reason I am so pleased to have come to Shimadzu is that they produce world-class mass spectrometers and I had access to the next-generation prototypes. Without such high sensitivity, I may never have thought of measuring such small mass differences.”

The meeting of the “eye” with which Nishimura studies the human body and the “eye” of the mass spectrometer that views proteins has achieved significant results. They watch over the bright path of proteomics.

A More Sensitive and Accurate View of Proteins

In 2002, Koichi Tanaka was awarded the Nobel Prize in Chemistry for the soft Laser desorption/ionization method that he invented in 1985. The mass spectrometer based on this method has

been significantly improved since the 1980s, particularly in terms of enhanced sensitivity and accuracy. Where instruments at that time could only detect the weight of 600 trillion protein molecules, today’s instruments are over a million times more sensitive and are able to detect just a few hundred million molecules.

Similarly, the accuracy has improved from the initial $\pm 0.1\%$ error to just 0.001% in the latest AXIMA Series instruments. These improvements now offer accurate, sensitive and rapid mass spectrometry. The success of the tryptophan method described before is attributed entirely to the performance of the AXIMA Series.

Tanaka states that he used the AXIMA Series to help confirm the ideas that Nishimura had been considering over a long period.

Protein analysis using these instruments has formed the basis of realizing the dream of disease diagnosis.

“The next step was to determine what type of system we could create using this method and the proper reagents to achieve good proteome analysis,” stated Tanaka.

Bedside Protein Analysis

Tanaka, who is once again able to work as a researcher and scientist, looked back over the research for which he was awarded the Nobel Prize: “At that time, I was concentrating on



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doing research every day. Even now, my days revolve around solid research. I achieved my dreams through this accumulated bulk of work. The most important thing is to maintain a balance between pursuing dreams and achieving results through steady fundamental and applied research.”

One of those dreams is research into sugar chains. As the name suggests, these are substances formed from sugars linking together like a chain. They play an extremely important role in a various protein functions. An estimated 30% of proteins function due to bonded sugar chains. Consequently, research into proteins



Koichi Tanaka

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Joined Shimadzu in 1983 after graduation from Tohoku University.

Awarded the Nobel Prize in Chemistry 2002 for the development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules.

Visiting Professor at Tohoku University, Kyoto University, Tsukuba University, and Ehime University

alone cannot provide greater understanding of life or human molecular mechanisms. Research into the sugar chains attached to the surfaces of proteins is important in understanding the phenomenon of life.

The analysis of sugar chains is extremely difficult and the existence of sugar chains was conventionally ignored when studying proteins. This situation is now changing, thanks to the AXIMA Series.

“Japan is the world leader in sugar-chain research. To further promote this research, I hope to aid other researchers by developing an analysis method for sugar chains,” explains Tanaka.

Another of his dreams is to use proteins to screen for disease. The ability to rapidly detect disease through protein analysis of blood samples would dramatically improve public health screening initiatives. The sensitivity, accuracy, and compact nature of the analytical instruments are the key to such research and development.

Tanaka added, “Protein analysis by mass spectrometry is extremely effective for screenings to determine the presence of disease. Abnormalities can be detected in the tiny amounts of protein present in minute samples of blood. I hope that protein analysis will become one means of health screening in the future.”

Work in the current topic, development of reagents for proteome analysis, should be one step along this path. In the near future, protein analysis may be as simple as blood pressure measurements are today.