Classic Paper Presentation

# The Origin of Macromolecule Ionization by Laser Irradiation

By Koichi Tanaka
On December 8, 2002

Atrayee Datta
31st August 2024

## 1. Introduction

When I was a student at university, my major field of study was electrical engineering. I did not specialize in chemistry in my studies at university, and my knowledge of chemistry overall was somewhat less than rich. Under those circumstances, upon entering the Shimadzu Corporation, I applied myself wholeheartedly to the study of chemistry to challenge and overcome a barrier in analytical chemistry considered at that time to be impenetrable. I am, of course, very pleased to receive the Nobel Prize and further, especially pleased that the Prize serves to acknowledge my efforts and results in the field of analytical chemistry, a field that while rather inconspicuous to the general population is indeed very important and useful to the world.

I am employed by the analytical instrument manufacturer, Shimadzu Corporation. I was assigned to a team charged with the task of designing and building a completely new mass spectrometer. The achievement of developing “a soft desorption ionization method for mass spectrometric analysis of biological macromolecules”, for which I was awarded this prize, was one aspect of that project. At that period in the 1980s, four of my colleagues, whom I will introduce shortly, in addition to myself, were each in charge of developing a part of the instrument. I was responsible for developing the sample preparation and ionization technologies. Success of the instrument would not have been assured unless the other technologies were suitably provided, even with an excellent ionization technology. First, I will briefly describe the progress of development of the technologies for the other components at that time, other than that of ionization.

# 6. Ionization Technology

Long before the 1980s, there had been investigations into the use of optical energy, not limited to laser light, to achieve ionization of organic compounds.[4] However, for the most part, this ionization consisted of stripping electrons from compounds in the gas phase.[5] Because this was limited to compounds that could be vaporized without being decomposed, very few compounds could be ionized. When a solid-phase organic compound was irradiated with a laser, the organic compound absorbed the laser light, which provided enough energy for desorption. Further, when the positive and negative charges of the particle are not in balance, it could be measured as an ion. However, in this case, since the laser light is absorbed directly into the analyte, the molecular bonds may be broken owing to the increased internal energy. In other words, there is an increased risk that measurement would occur in the decomposed state (Figure 11). Because we wanted to measure the undecomposed, intact molecular ion, this was unsatisfactory.

## 6.1 Rapid-Heating Technology

In the 1980s, “desorption to gas phase by rapid heating” gained attention through the efforts of several groups that had obtained some encouraging results. These include Franz Röllgen[6,7] of Germany, Mamoru Ohashi[8] of Japan, and Louis Friedman,[9] Don Hunt,[10] Burnaby Munson,[11] and Robert Cotter[12] of the USA. A simple description of the principle is depicted in Figure 12. In short, if a given compound AB is heated, it is assumed that AB will be released to the gas phase as intact AB as well as fragmented A and B [Eq. (5 a, b)]. Respectively applying the well-known

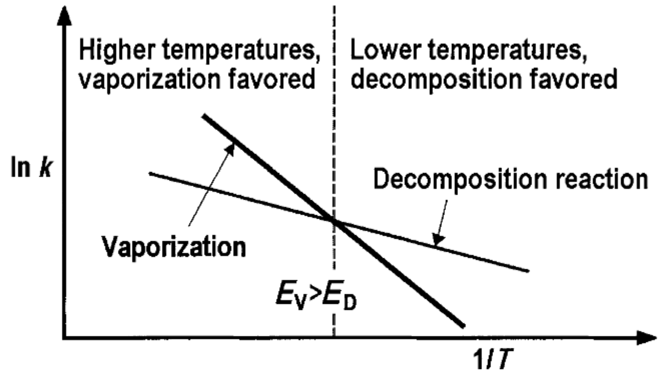


**Figure 12.** Temperature dependence of vaporization versus decomposition.[9]

$$\text{Vaporization}: \quad \mathrm{AB} \xrightarrow{K_V} \mathrm{AB} \tag{5a}$$

$$\text{Decomposition}: \quad \mathrm{AB} \xrightarrow{K_D} \mathrm{A} + \mathrm{B} \tag{5b}$$

For compounds that are thermally labile and decompose easily, the rate constant for decomposition $k_D$ is larger than that for vaporization $k_V$ at low temperatures. Because the activation energy for vaporization $E_V$ is higher than that for decomposition $E_D$, the slope of the vaporization reaction $k_V$ in

Figure 12 ($\ln k$ versus $1/T$) is steeper than that of the decomposition reaction. If this relationship is always correct, at high temperature, where $1/T$ is small, vaporization should be favored. In other words, reaching the highest possible temperature as quickly as possible should provide a high degree of vaporization.

### 6.1.1. *Use Of Ultrafine Metal Powder*

To perform this rapid heating, various heating methods were devised.[9] However, a high enough temperature could not be achieved quickly enough to obtain intact vaporization of macromolecules, such as proteins. Since the time width of a pulse laser itself is between a nanosecond to several microseconds, rapid heating would seem possible, that using a focused laser beam would generate energy at high density and high speed. However, this would require a medium to enable the conversion of the irradiating photons into thermal energy.

In Japan at that time in the 1980s, ultrafine metal powder (UFMP) received much attention in the field of metallurgy owing to its usefulness in the efficient production of alloys, and was referred to as “Japanese Powder”. UFMP, as its name indicates, is an extremely fine metallic powder with particle diameters measuring a few tens of nanometers. The particle diameter of UFMP is about the same as a wavelength, thus when compared to bulk metal, there is an increasing possibility that scattered light could be taken up internally

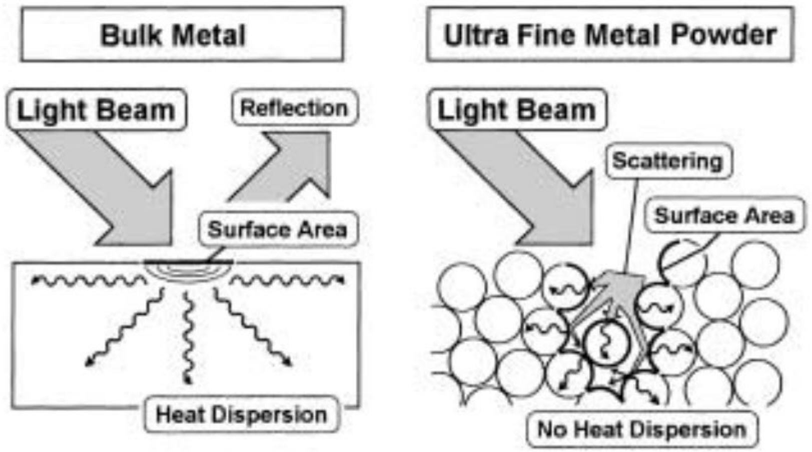


***Figure 13.*** Differences between light absorption by bulk metal and ultrafine metal powder.

Moreover, the possibility that thermal energy would be dispersed and lost decreases owing to the interparticle spaces.

Mixing UFMP with an organic compound should allow the laser light to be efficiently absorbed into the UFMP, and to achieve a high UFMP temperature very quickly without the dispersion and loss of heat. This would efficiently achieve heating of the sample in the mixture. It was Mr. Yoshikazu

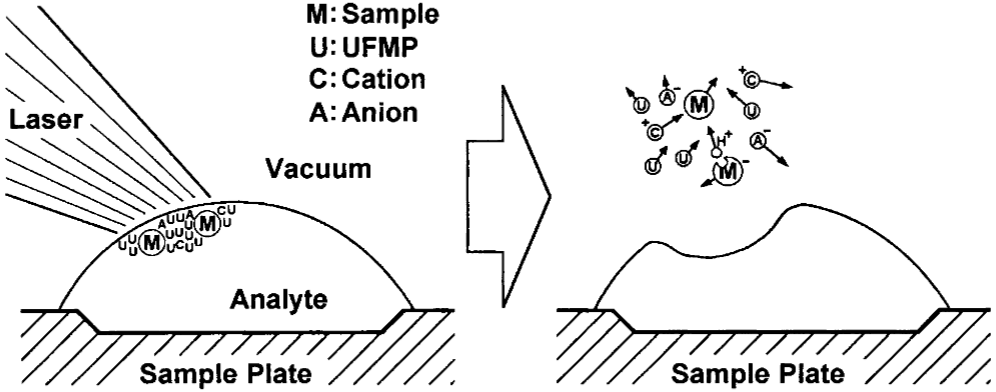


***Figure 14.*** Molecular-ion desorption by using the UFMP matrix.

With the use of this matrix, the molecular ions from many organic compounds that could not be measured previously could now be measured by mass spectrometry. However, according to our experimental results, this technique could not be applied to compounds with molecular weights in the tens of thousands.

### 6.1.2. *Glycerin Matrix Technology*

At that time in the 1980s, the fast atom bombardment (FAB) MS method was widely used to achieve ionization in thermally labile compounds. The FAB method was developed by Donald Sedgwick and colleagues,[14] and David Surman and John Vickerman[15] of the UK, and involved accelerating neutral particles, such as xenon, at high speed so as to collide with the sample and generate ions. In this method, the solid sample was maintained in a liquid state by using glycerin (glycerol) as a matrix (Figure 16).It is thought that since glycerin is a liquid at room temperature, it releases the solid sample from its crystalline state to dissolve in the liquid, thereby assisting ionization. We used glycerin as a matrix, just as it was used in FAB, but because the $N_2$ laser wavelength (337 nm) that we used was only slightly absorbed into the glycerin, no significant effect was seen on the enhancement of molecular-ion generation for organic compounds. However, this matrix was useful in improving reproducibility owing to its effectiveness in providing a uniform mixture

### 6.1.3. *UFMP–Glycerin Mixture Technology*

Here it can be said that I had come up against a brick wall. Soon after that, while preparing many trial-and-error UFMP suspensions, changing the organic solvent and concentration repeatedly to obtain even the slightest improvement in data, I committed a monumental blunder, which was followed by a series of fortuitous, arbitrary decisions. I was using ordinary acetone as a UFMP suspension, and one day, by mistake, 1) I used the glycerin instead of the acetone. Reasoning that it would be wasteful to discard the expensive UFMP, 2) I decided to use the “ruined” preparation as the matrix solution. Because glycerin gradually evaporates in a vacuum, I knew it would eventually disappear. However, instead of waiting, 3) I decided to speed up vaporization of the glycerine by continuing to irradiate the laser. On top of that, because I wanted to see the result as soon as possible, 4) I was monitoring the acquisition of the TOF spectrum. In this way, only after at least four factors fell into place could I observe a phenomenon never before seen.

I think that the sample first measured was vitamin $B_{12}$. I have tried recently to locate the original spectrum, but unfortunately I could not find it. Because vitamin $B_{12}$ itself can absorb laser light with an extremely high degree of efficiency, up to then, only fragment ions could be measured with high sensitivity. However, by using the mixed UFMP–glycerin matrix, at the position where unfragmented ions (molecular ions) should have appeared, peaks which seemed to be noise were observed. No matter how many times I performed the measurement, these peaks were present. Molecular ions were observed even after changing the sample, and therefore must be the molecular ion of vitamin $B_{12}$.

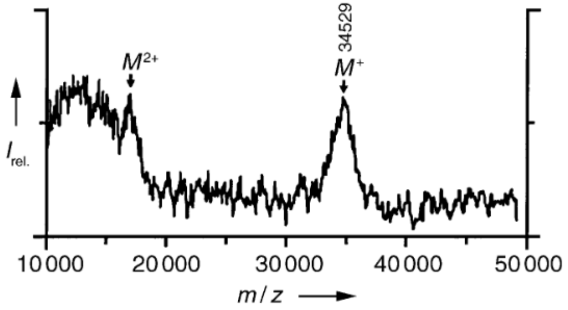


***Figure 17.*** Molecular-ion measurement of carboxypeptidase A (molecular weight ≈ 35k Da).

After that, as a result of progressing to higher mass ions while optimizing the concentration, laser power, and other parameters, I was able to measure an ion having a molecular weight of 35 000 Da in 1985 (Figure 17) and in 1987 an ion cluster having a mass number exceeding 100 000 Da (Figure 18). The patent application for this ionization method was submitted in Japan in 1985, and was subsequently issued in 1993 (Patent number: JP 01769145).

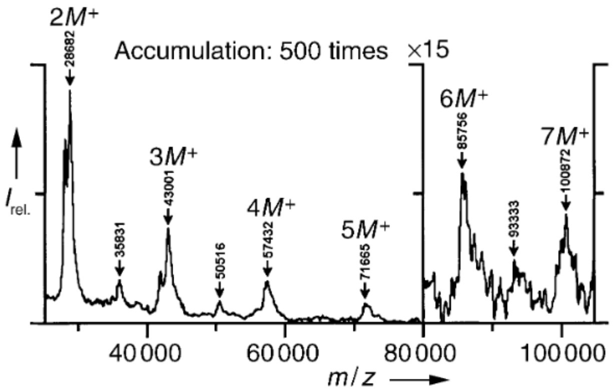


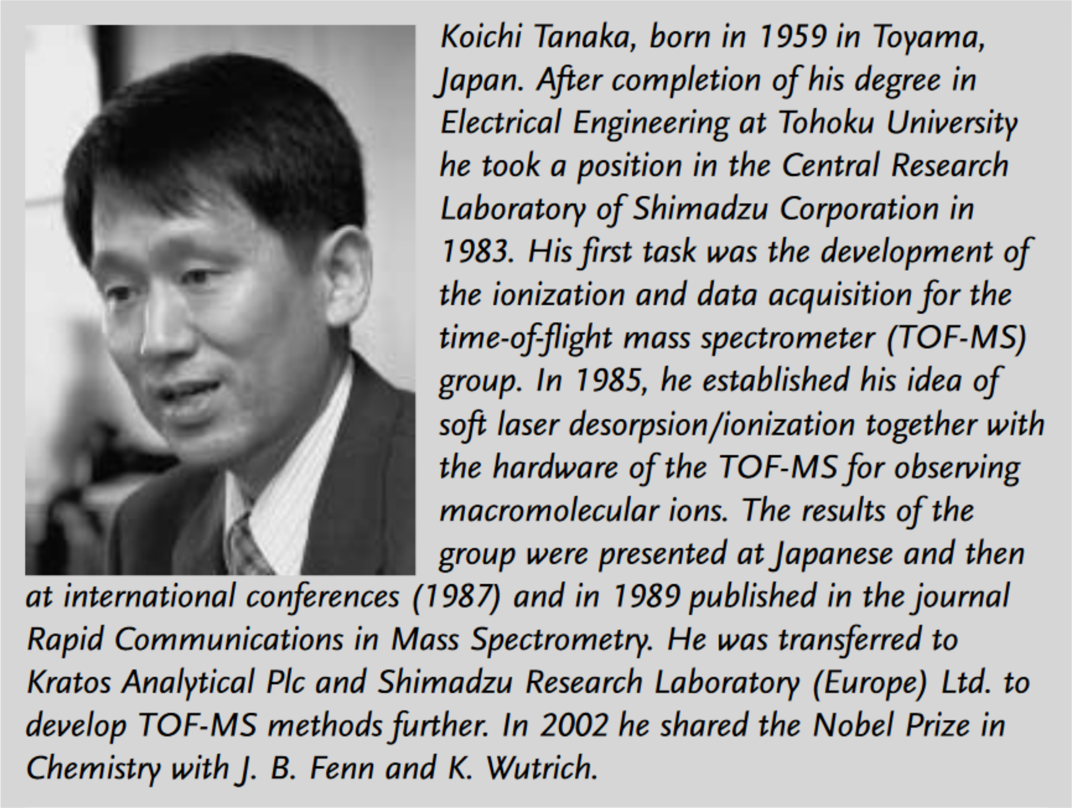
***Figure 18.*** Molecular cluster-ion measurement of lysozyme (molecular weight = 14 306 Da).

### 6.2. Why Did The Macromolecule Become Ionized Without Decomposing?

Use of UFMP conveniently allows high temperatures to be reached at high speed. However, when this is mixed with a typical organic compound, it is difficult to achieve sufficient mixing. Even when viewing the mixture in an electron-microscope-generated photograph, the lack of uniformity is obvious (Figure 19, top). When glycerin is added to the mixture, however, the uniformity becomes evident (Figure 19, bottom). Moreover, glycerin may also serve to release the sample from its crystalline state. The ability to measure molecular ions from organic compounds even exceeding a molecular weight of 10000 Da may indeed be due to these effects (Figure 20).

Herein I have suggested the principles by which laser-light irradiation is able to generate huge molecular ions. However, these principles are not necessarily correct because they have not yet been fully proven scientifically. Even with the knowledge of all the developments in MALDI (matrix-assisted laser desorption/ionization) technology that followed my discovery, I cannot yet claim to have correctly grasped the theoretical principles behind all of these phenomena. However, I am an engineer, an engineer employed in a business enterprise. So even if the principles are unverified, their application takes priority if they are useful and practical.

# Biography



*Koichi Tanaka, born in 1959 in Toyama, Japan. After completion of his degree in Electrical Engineering at Tohoku University he took a position in the Central Research Laboratory of Shimadzu Corporation in 1983. His first task was the development of the ionization and data acquisition for the time-of-flight mass spectrometer (TOF-MS) group. In 1985, he established his idea of soft laser desorpsion/ionization together with the hardware of the TOF-MS for observing macromolecular ions. The results of the group were presented at Japanese and then at international conferences (1987) and in 1989 published in the journal Rapid Communications in Mass Spectrometry. He was transferred to Kratos Analytical Plc and Shimadzu Research Laboratory (Europe) Ltd. to develop TOF-MS methods further. In 2002 he shared the Nobel Prize in Chemistry with J. B. Fenn and K. Wutrich.*