The history of the mass spectrometry of peptides and proteins in the USSR

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A personal recollection of the development of mass spectrometry of peptides and proteins in the Soviet Union is presented.

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Introduction

Many historical details relating to the development of the various fields of life science in the Soviet Union are little known abroad. The new generation of Russian scientists are also not so familiar with this history. These circumstances stimulated me to recollect the process of development of mass spectrometry of peptides and proteins in the USSR which may be of interest to the mass spectrometry (MS) community.

History

1950s

In the USSR, organic mass spectrometry began in the early 1950s with the quantitative analysis of natural petroleum products.[1] Before that time, mass spectrometers were only used successfully for special physicochemical research[2] and the analysis of isotopes.[3]

The first attempts to use mass spectrometry for the study of natural products other than petrochemicals came a decade later.[4] The mass spectrometry of bioorganic molecules began at the Institute of the Chemistry of Natural Products, USSR Academy of Sciences in Moscow, which was founded in 1959. The goal of this institute was the acceleration of research in bioorganic chemistry. Due to excellent financial support from the USSR Government and good managerial leadership, this institute very soon held the leading position in the study of physicochemical biology in the USSR.

1960s

The origin of mass spectrometry of natural compounds in the USSR was certainly stimulated by its early successful application in some leading laboratories elsewhere in the world. More specifically, a powerful stimulus for the beginning of this research in the USSR came from the results of mass spectral analysis of some alkaloids and short peptides reported by K. Biemann at the 1960 IUPAC meeting in Australia.[5]

The director of the Institute, M.M. Shemyakin, the deputy director N.K. Kochetkov and other leading scientists of this new Institute, became the initiators of the use of mass spectrometry for the analysis of natural compounds in the USSR. Projects to use mass spectrometry for analysis of carbohydrates were initiated by N.K. Kochetkov[5] and mass spectral studies of peptides and proteins was pioneered by M.M. Shemyakin.[4] In time, organic mass spectrometry became widely used at many other institutes in the USSR.[6]

I joined the Mass Spectrometry laboratory at the Institute of the Chemistry of Natural Products (now known as the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences) in 1963. I obtained my doctoral degree with N.S. Wulfson and V.A. Puchkov as supervisors in 1966, working on problems associated with the MS analysis of natural and synthetic depsipeptides. In 1969, the mass spectral laboratory was transfered to the Department of Instrumental Methods of Analysis. I was appointed as the head of the MS group. I retired from the Institute in 1994 and was invited to work for Aspen Research Corporation, a private company in the United States.

As already mentioned, the mass spectrometry of peptides originated and, until 1985, developed almost exclusively at the Institute of Bioorganic Chemistry. Thus, the most significant part of my work in MS in the USSR was very closely connected with peptide analysis.

My involvement with the mass spectrometry of peptides began from the study of atypical peptides under electron

impact. I worked on problems associated with the development of techniques and methodologies for mass spectral analysis of peptides and proteins, the testing of commercial mass spectrometers produced by the leading instrument companies around the world and the development of mass spectrometry equipment in the USSR. This work is not widely known to the new mass spectrometric generation.

The following story is told from the perspective of an active participant in the Institute, whose history closely paralleled the evolution of mass spectrometry of peptides and proteins in the USSR.[7]

This paper is not concerned with the details of the research. Rather, it is a personal recollection of the development of the mass spectrometry of proteins in the USSR and its interrelationship with world science. In the interest of offering a coherent picture, there has been no attempt to present all of the facts. Many individuals have contributed to this process. The author apologizes for not being able to acknowledge the contributions of everyone, due to the purpose and scope of this article.

In 1963, I began to study electron impact (EI) mass spectrometry of some cyclic depsipeptides in a group headed by V.A. Puchkov.[8]

The cyclodepsipeptide ionofores are the atypical natural peptides built from amino and hydroxy acid residues combined via the amide and ester bonds. Many antibiotics, toxins and alkaloids belong to this class of compounds.

At that time, this class of compounds was extensively studied in the Laboratory of the Chemistry of Antibiotics headed by M.M. Shemyakin. Many of the new compounds were synthesized and the chemists were very interested in simple and reliable methods for analyzing these compounds.[9,10]

The molecular weights of some of these compounds were greater than 1000. Mass spectral analysis was very difficult using the only instrument available in the laboratory. That mass spectrometer was a magnetic sector instrument that had been manufactured in the USSR. That instrument, called the MX-1303, possessed a heated-reservoir inlet designed for the analysis of petroleum products. The instrument had an upper mass limit of ~ 600 amu and a resolution of 450.[11] The scanning process was slow and difficult to reproduce. The instrument was suitable for the analysis of small volatile cyclodepsipeptides but was quite unsuitable for the analysis of their high molecular weight homologs. The first attempts to analyze large cyclodepsipeptides were unsuccessful. The thermal instability of these compounds in the heated reservoir led to thermal degradation. The mass spectrometric analysis of these compounds only became possible after using a glass direct-inlet probe designed and constructed by an aspirant of that time, A.M. Zyakun, who had a particularly well developed engineering talent.

The study of cyclodepsipeptides was the first organic mass spectrometry research for all those taking part in this work. Our reading of the books written by J.H. Beynon,[11] K. Biemann,[12] F.W. McLafferty[13] and later on by C. Djerassi and colleagues,[14] was our organic mass spectrometry education.

The most valuable result of our study was the understanding that the amino(hydroxy) acid sequence in depsipeptides could be determined by mass spectrometry.[15]

In 1965 M.M. Shemyakin began to take an interest in the mass spectrometry of peptides. The goal of this research was the application of mass spectrometry for protein structure elucidation.

Once again, organic mass spectrometers available in the USSR in 1965 were unsuitable for peptide analysis. The purchase of a more suitable mas spectrometer abroad was complicated by a number of political and economic issues taking place in the USSR at that time. The technical feasibility of developing a domestic MS for peptide analysis was questionable. This situation forced the Institute to think not only about purchasing foreign instruments but also about developing MS jointly with domestic manufacturers.

In 1965, an RMU-6D (Hitachi, Japan) was installed at the Institute. A special group, which included chemists and mass spectroscopists, started to work on the problems of mass spectrometry of peptides with this new tool. The leader of this work was A.A. Kirushkin.

Many hundreds of peptides with varied structures were synthesized and studied by EI mass spectrometry.[16] For the first time, we were working with a commercial instrument designed for the routine mass spectrometric analysis of large nonvolatile organic molecules. Synthesized peptides were analyzed as the N-decanoylpeptide methyl esters.[17] For mass spectrometry, arginine residues in peptides were converted into either N-pirimidylornithine or N-acylornitin.[18] The volatility of sulfur-containing peptides was greatly enhanced by their desulfurization.[19]

The application of EI mass spectrometry for structural analysis of peptides depended mainly on the volatility of the peptide derivatives. However, acylpeptide esters higher than decapeptides do not usually give interpretable mass spectra.

In 1967, the Laboratory of Protein Chemistry was established at the Institute. Shemyakin's disciple, Yu.A. Ovchinnikov, was appointed Chief of this laboratory. He, too, was interested in the application of mass spectrometry for determining the primary structure of proteins.[20] In 1968, a GC-MS 900 (LKB, Sweden) instrument was installed at the Institute.

In 1969, a method of determining the amino acid sequence in peptides by EI mass spectrometry was developed.[21] About this time the Laboratory of Antibiotics and the Laboratory of Protein Chemistry started a joint study of the primary structure of cytoplasmic aspartate aminotransferase (AAT) from pig heart. That was the first research of protein structure in the USSR directed by M.M. Shemyakin. The chemists involved in this research had little experience in structural protein chemistry. In the course of this work, they enthusiastically learned the classical methods of protein chemistry and tried to find ways to use mass spectrometry rationally in this research.[22,23]

As already mentioned, EI-MS could not, at that time, be applied to the analysis of peptides containing more than ten amino acid residues. The peptides from tryptic hydrolyzate of AAT often contained more residues. It was proposed to shorten these peptides prior to mass spectrometry by a combination of chemical and enzymatic methods. The shortening from the N-terminus was provided by the Edman procedure and shortening from the C-terminus by using carboxypeptidases.[24] Also, the use of dipeptidyl aminopeptidases for exhaustive digestion, in order to perform GC-MS analysis of the resultant dipeptides, was proposed for the sequencing of the tryptic peptides.[25]

1970s

In 1970, M.M. Shemyakin passed away. His follower, Yu.A. Ovchinnikov, was appointed the new director and headed protein research at the Institute.[26]

As before, the purchase of modern scientific equipment was one of the Institute's most important goals. In 1970, a high-resolution mass spectrometer (MS-902, AEI, UK) was installed. It was the first instrument equipped with a computer-based data system.

With this new equipment, the research into protein chemistry successfully continued and expanded.[27]

In the early 1970s, the complete amino acid sequence of AAT was determined. At that time, ATT, which contained 412 amino acid residues, was the third largest protein whose structure had been determined anywhere in the world.[18] This work was carried out by the classical methods of protein chemistry.[28]

However, to elucidate overlapping peptides for determining the complete amino acid sequence, AAT was digested with thermolysin and then MS was used to analyze the products of hydrolysis.[29,30] 128 peptides were found in the hydrolyzate. MS was applied to determine the structure of 92 peptides that contained from two to six amino acid residues. All peptides were analyzed after conversion into N-decanoylpeptide methyl esters. Thus, 60% of the amino acid sequence of aspartate aminotransferase was determined by mass spectrometry. The results of this work were first reported at the 1974 Third All-Union Symposium on Chemistry of Peptides and Proteins in Kiev but were not widely known to the international scientific community until now.

The use of EI-MS for the elucidation of the primary structure of AAT proved to be the first and the last example of such research at the Institute of Bioorganic Chemistry. The passionate fight between the leaders of the project was the main reason. A.A. Kirushkin, who was supportive of the collaboration of protein chemists and mass spectrometrists, lost this argument to others who successfully used classical methods for determining the primary structure of AAT. They concluded that the application of EI-MS in the structural protein research was inefficient.

Sometime later, the effective application of EI-MS in a protein study by H. Morris, K. Biemann and others showed that conclusion to be premature.[31,32]

In time, major contributions from mass spectroscopists have helped convince protein chemists that MS is, in fact, a useful tool for peptide analysis.

Many different synthesized peptides have been analyzed by MS, both in the USSR and elsewhere.[32-36] Also, the detailed study of the fragmentation pathway's of N-methylated cyclodepsipeptides was carried out using high resolution mass spectrometry.[37] Computer programs were developed for the determination of the sequence of N-decanoylpeptide methyl esters from high-resolution mass spectra.[38]

The application of the metastable defocusing and database assisted density interpretation (DADI) techniques allowed the structure of N-decanoylpeptide methyl esters and natural ornithinolipids to be successfully studied by MS.[39,40]

Jointly with the Institute of Cardiology, a new method of isotope analysis of biologically-active compounds was found. Small amounts of the biological materials were burned in helium in the presence of thermally-stable oxidizers. The products of complete oxidation (N_2, CO_2 and H_2O) were eluted from the reactor into a GC with helium. After separation, the gases were analyzed by an isotope mass spectrometer, the MI-1305 (USSR).[41]

The study of the N-decanoylpeptide methyl esters was carried out on a high-resolution instrument, the MC-3301, which employed a CI ion source. This instrument was developed for the Institute at the Special Designing Bureau, USSR Academy of Sciences in 1976.[29]

By 1977, the magnetic-sector mass spectrometer, a MAT CH-5DF, with a field desorption ion source, and a quadrupole GC-MS, a MAT-44, with desorption chemical ionization (both from Varian, Germany) were installed at the Institute.

The field desorption mass spectrometer was used for analyzing mixtures of intact dipeptides and synthesized glycopeptide components of antitumor preparations, obtained from cell walls of *Lactobaccilus bulgaricus*.[42] The results of this work were presented at the 1977 Fourth All-Union Symposium on Chemistry Proteins and Peptides in Minsk. In spite of some progress in peptide analysis, this instrument was not intended for routine work in protein chemistry.

The fully computer-controlled MAT-44, with real-time data handling, became the most convenient instrument for the routine analysis of amino acid derivatives and short peptides.[43,44] Our use of the MAT-44 showed the many advantages of using a quadrupole instead of a magnetic-sector mass spectrometer for routine analysis of peptides.

So we see that during the 1970s, different commercially-available ionization and desorption/ionization techniques were being applied for peptide analysis at the Institute. This work showed that none these techniques was versatile enough for the task. Only with special instrumentation were selected laboratories able successfully to conduct analyses of peptides originating from digested protein. It

became evident that it was necessary to develop new mass spectral techniques for protein chemistry.

1980s

In the early 1980s, the introduction of the fast atom bombardment (FAB) source adapted to all kinds of mass analyzers, made organic mass spectrometry the universal tool in protein chemistry.[45] The use of FAB-MS made the protein chemists' routine "weighing" of intact oligopeptides practical at every stage of protein degradation.

In the USSR, G.D. Tantsyrev and his coworkers in the Institute of Chemical Physics developed a FAB ion source for MS of organic materials in early 1970s. But the underdeveloped MS technology did not allow the development of a useful instrument for analysis of large peptides.[46]

The commercial tandem mass spectrometer MS-50TC (Kratos, UK) with a FAB ion source was not installed at the Institute until 1987. The appearance of this instrument simplified routine mass determinations for most of the very large intact peptides being studied at the Institute.

Before that, MS analysis of large oligopeptides in the Institute was only possible after degradation. An approach based on conversion of the degradation products into N-dansylpeptide methyl esters, followed by HPLC and EI-MS, was proposed in 1981.[47,48] This method was applied to the analysis of glycopeptide (MW 10,000) from blastolysin, an antitumor bacterial preparation isolated from the *Lactobacillus bulgaricus* cell wall.[43]

During the 1980s, the Institute of Bioorganic Chemistry initiated the development of a mass spectrometer for the analysis of peptides directly from solution. Utilizing an electrospray source coupled to an retarding grid system with a Faraday cage detector to determine the mass of some intact protein ions was pioneered by M. Dole and co-workers.[49] Use of a mass spectrometer with a high voltage post-acceleration ion detector to determine the mass of ions in Dole's experiment was further performed by Beuhler and Friedman at the Brookhaven National Laboratory.[50,51] This showed the practical possibilities of coupling ES and MS for peptide analysis. The Designing Bureau of the Academy of Sciences worked on the development of this type of instrument.

The first description of a prototype of the instrument for the analysis of peptides in solutions was published in 1984.[52]

The developers described this instrument, called LC-MC-3303,[53] as a "mass spectrometer for the extraction of dissolved ions at atmospheric pressure".

Actually, it was the first magnetic-sector mass spectrometer with an electrospray ionization (ESI) source. The application of this instrument for the analysis of the peptide hydrolyzates was demostrated in 1985.[54] The ESI source for this instrument produced mostly singly-charged ions, which simplified mixture analysis.[55]

In 1987, the ESI source developed for the magnetic-sector instrument was adapted to orthogonal injection ToF-MS with a Mamyrin reflectron by A.F. Dodonov and coworkers from the Institute of Chemical Physics, USSR Academy of Sciences. They were able to observe a multiply-charged molecular ion of insulin.[56]

A comparison study of ESI and other ionization techniques—EI, CI, FD and FAB—was carried out on the mixtures of cystine-containing peptides in 1986.[57]

In 1987, O.S. Reshetova, while collaborating with the protein group headed by V.M. Lipkin at the Institute of Bioorganic Chemistry, used this magnetic-sector ESI mass spectrometer for peptide mass mapping in the tryptic and chymotryptic hydrolyzates of cyclo-GMP phospho- diesterase γ-subunit.[58] This was the first time ESI mass spectrometry was used as a complementary method for the verification of the results of sequence determination by chemical methods.[59] This work was published in Russian and not cited until now in the special reviews.

The development of ESI magnetic-sector MS in Leningrad (now St Petersburg) extended the boundaries of the use of mass spectrometry for peptide and protein analysis outside of the Institute of Bioorganic Chemistry.

1990s

O.S. Mirgorodskaya and his coworkers in St Petersburg[60–62] pioneered the application of the prototype of the ESI magnet-sector MS to the solution of some problems in medical enzymology and to comparison studies of hemoglobin. In 1993, Mirgorodskaya, collaborating with ToF-MS developers from the Institute of Chemical Physics Russian Academy of Sciences, used, for the first time, an orthogonal-injection reflecting time-of-flight mass spectrometer (ToF-MS) with electrospray ion source for peptide mass mapping.[63]

The last major event in the history of mass spectrometry of proteins in the USSR was the development of the commercial plasma desorption ToF-MS for the express analysis of protein compounds in 1986–1990.[64–66] The development of this instrument stemmed from the work of Mcfarlane and his coworkers on PDMS in the USA. The instrument was developed at Sumy (now Joint-Stock Company SELMI, Ukraine) in collaboration with the Institute of Bioorganic Chemistry.

In conclusion, I would like to note that the development of mass spectrometry of proteins in the USSR stemmed from active contact with leading foreign researchers involved in the mass spectrometry of biopolymers. Over a period of time, guests of the Institute of Bioorganic Chemistry were Professors H. Nau (West Germany), H.R. Morris (UK), A. Burlingame (USA), B. Sundqvist (Sweden), P. Roepstorff (Denmark), K. Standing (Canada), Y. Le Beyec (France) and others. Good relationships with Kratos and Varian, leading to the production of mass spectrometers at that time, was also crucial to our efforts and work.

Looking back on my work in mass spectrometry in the USSR, I believe that the limited success of the applications of mass spectrometry for structural analysis of peptides and proteins can be explained in two main ways. First, the absence of competition between the scientific centers using mass spectrometry in peptide and protein research and domi-

nation of the Institute of Bioorganic Chemistry in the field. Second, by the attempt to develop mass spectrometers for experts and not for chemists and biologists. The USSR did not have sufficient social and economical infrastructure and high technological production for development, commercialization and marketing of customer-oriented broad-based mass spectral equipment. This is why many of the young Soviet scientists who took part in the development of the instruments mentioned in this story readily adapted to instrumental laboratories abroad and have developed new mass spectrometers. Such scientists include A. Verentchikov (now working for PerSeptive BioSystems, Inc., USA) who developed a Russian ESI source, I. Chernushevich (now working for PE SCIEX, Canada) who developed the Russian reflecting orthogonal injection time-of-flight mass spectrometer, P. Bondarenko (now working for Thermo BioAnalysis Corporation, USA) who developed the Russian commercial plasma desorption time-of-flight MS, and several others who are successfully working in instrument development abroad. Modern mass spectrometry technology has become a very attractive field of endeavor for many well-educated physicists and physiochemists from the former USSR.

Thus, what started as a project important to the acceleration of USSR biological research, has significantly contributed to the advancement of the world's biological sciences and to the general development of mass spectrometry.

Conclusion

I was fortunate to have played a role in the development of mass spectrometry of peptides and proteins in the Soviet Union for about 30 years of my employment at the Institute of Bioorganic Chemistry, USSR Academy of Sciences. The long development of this new field of science has been characterized by the active collaboration of many dedicated scientists and engineers who developed new methods and built new instruments for protein study. I realize that there were many men not mentioned in this paper with whom I collaborated for a long time and to whom I am deeply indebted. In particular, I would like to underline the leading role of the institute founder M.M. Shemyakin in the organization and leadership of the mass spectral study of peptides and proteins and for his ability to obtain special government funds for carrying out that project. Shemyakin's disciple, Yu.A. Ovchinnikov, who became director after his teacher's death, was able to reach a top political position in the state and obtained much more money, not only for protein and other biopolymer research at the Institute, but also for the progress of life science in general. Due to the presence of dedicated scientists and good financing, it was possible for the Institute to develop ambitious and expensive projects in bioorganic chemistry which produced world-class results.

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