MS analysis of polypeptides produced by hydrolysis of collagen: from technology to interesting discovery?

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Continuously an increasing interest in polypeptides is observed. They are investigated in details and applied in various areas of chemical and food industry, medicine, pharmacology, cosmetology, or light engineering [1–8]. Polypeptides can be produced on a large scale by a variety of methods, including hydrolysis of collagen [9, 10]. Recently, we have published proecological and energy-saving technology of polypeptides production from the above raw material, that means from the minced animal hide and skin scraps (collected at a slaughter of cattle and pigs). It was developed and commercially implemented in the Chemical Cooperative Company "Żelatyna" located in Słupsk, Poland (currently: LOTON) [11]. The particularly important problem in this technology is analysis of the hydrolysate, that means determination of the molecular mass of polypeptides. We were able to elaborate fast and convenient analytical method with the use of mass spectrometry (MS). It allows to control 'on-line' the hydrolysis process, as well as to identify polypeptide fractions in the final product. MS spectra were recorded using so-called soft ionization techniques (electrospray, photospray, photoionization under atmospheric pressure). The idea and details of this method were published in *Przemysł chemiczny* [12].

The elaboration of the above method and its utilization in our technology was possible due to observation that *polypeptide molecules during MS measures are always charged approximately 50 times – independently of their molecular mass*. These unexpected results (the observed regularity of multiple ionization) were confirmed by electrophoresis [12], which is routinely used in analysis of peptides and proteins. In fact, the conclusion concerning the same level of multiple ionization was formulated on the basis of correlation between results obtained by MS and by electrophoretic method. In MS spectra there are series of bands in the low *m*/*z* range; *m*/*z* = 400–2500 (see example in Fig. 1a). Their remarkable mutual similarity with characteristic broad peaks distribution envelope in the spectrum is noticeable.

Spectra were congested because of the large population of ions measured. For example, in the range 43–44 kDa (the real fraction; see ref. [12]) potentially one can expect one thousand polypeptide chains; each of them could be multiple ionized (in agreement with Gaussian distribution [13] for series of peaks corresponding to sequential charges; $z_{av.} = 50$). Taking into account the range for the charge $z = z_{av.} \pm 10$ ($\Delta z = 20$), we will observe $n = 1000 \times \Delta z =$ 20000 ions for the chosen fraction of polypeptides. Additionally, each ion has characteristic isotope pattern, seen very well for the large molecular masses; *e.g.* for *M* = *ca* 40 kDa seven isotope ions (within the peak envelope) are easily observed. This gives 20000 × 7 = 140000 ions. Interpretation of such a 'broad peak' is practically impossible, especially in the case of interference with other neighbouring 'peaks'. However, the maximum of the curve can be determined; which is called average *m*/*z*.

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We found that all the maxima observed in the spectrum fit very well to average ionization step *z* = *ca* 50+, which is a good correlation of this tendency to multiple ionization with electrophoresis studies [12] and with data obtained by optical method (light scattering measurements) [11, 14]. That means, all the polypeptide chains formed in the hydrolysis process of collagen (independently of their molecular mass) are charged during the MS measure *ca* 50 times. This allowed to identify fractions of diverse molecular mass, mainly from the range 28–82 kDa.

In conclusion, if the hypothesis concerning ionization step is correct, then the shape of the bands for the large population of ions is a result of: (**a**) different length of polypeptide chains, (**b**) various multiple ionization for each molecule (representing approximately Gauss distribution), (**c**) presence of isotope ions, and (**d**) interferences with neighbouring peaks (originating from other fractions). In the spectra of product formed at the end of hydrolysis (Fig. 1b), most of the peaks are in the range *m*/*z* = 700–1500 ($[m/z]_{av}$ = *ca* 950). It corresponds to *M* = 35–75 kDa. Series of signals under the slope more far away from average *m*/*z* can be a result of circumstances described in items (**a**)–(**d**).

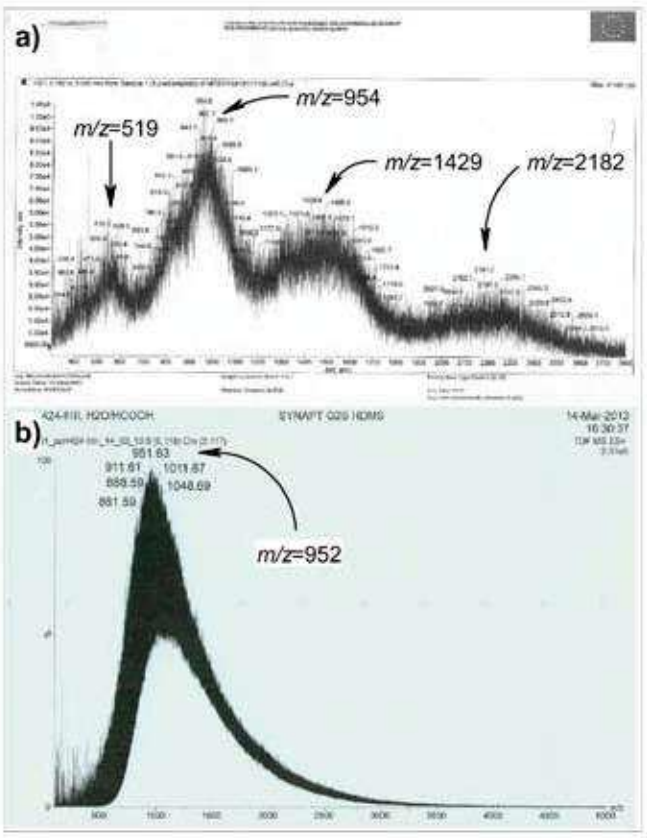


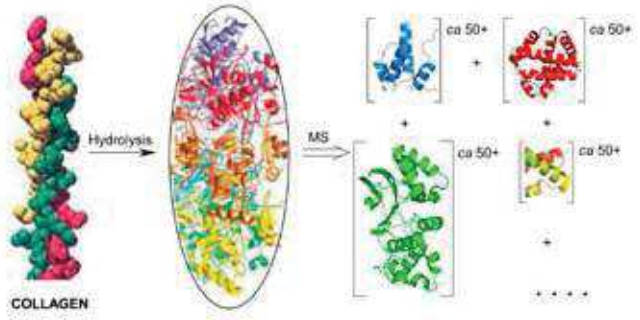
Fig. 1. MS spectra of polypeptide fractions: (a) the beginning step of hydrolysis of collagen (ESI turbo-spray technique), (b) spectrum of the selected fraction (ESI technique); for experimental details – see [12]. The maxima *m*/*z*×50 approximately correspond to average values of molecular mass of the polypeptides obtained.

Protonation of polypeptide units still remains an open research question. Although polypeptides have tendency to form multiple charged ions [15], commonly observed during the MS-ESI measurements, the preference to expose for protonation approximately 50 groups has so far been almost unknown. Especially intriguing is that molecules of different molecular mass had the same charge. In the literature, presenting MS spectra of peptide assemblies, usually correlation between the number of protons attached and the molecular mass of the chain can be found (spectra recorded with MS-ESI technique). For smaller molecules, this correlation is strongly-dependent on the number of basic amino acids in their structures. Addressing this problem probably requires undertaking more complex studies, involving MS investigations for single polypeptide strands. In this article, on the basis of MS data (obtained in our laboratory and those described in the literature), as well as the knowledge on the formation and the structure of collagen, the initial discussion concerning this observation is attempted.

First, in the literature there are examples of MS spectra for this type of compounds, in which high multiple charging was observed (transferrin: mainly 52+/54+ [16], α-foetal protein (a glycoprotein present in foetal serum): *ca* 50+ [17], bovine serum albumin (BSA): average 45+ [15]). It can be a region of upper limit of ionization level for such molecules (z = *ca* 50+). However, the conclusive answer probably should come from the structure of collagen, in which there is an unprecedented arrangement of amino acids that make it up [18]. This is almost unknown in other proteins.

Secondly, collagen mainly consists of repeating triads of amino acids: glycine-proline-hydroxyproline. Always, in this regular sequence every third position in polypeptide chains is occupied by glycine. However, instead of two remaining residues (together, *ca* 30% in collagen) small amounts of hydroxylysine (and some other amino acids) can be found in these triads. It is characteristic that hydroxyproline practically does not appear in proteins (except this one) or is present sporadically. There is no genetic codon for its insertion into a growing protein. The collagen molecule is first assembled mainly with proline. Then some proline residues are oxidized to hydroxyproline (in post-translational process catalyzed with hydroxylases in the presence of vitamin C). The deficiency of the latter causes scurvy due to inability of the organism to make collagen.

Third, polypeptide chains of collagen due to mutual interactions, have tendency to adopt well-defined conformations. The three long intertwined left-twisted strands (built-up of approximately 1000 amino acids) spontaneously wrap around one another into subunits known as tropocollagen. It has exhaustively packed right-handed triple-helical shape. The end-to-end and side-to-side bonded tropocollagen molecules assemble to form collagen fibrils [18, 19]. The presence of hydroxyproline, in conjunction with proline, results in their very strong coiled structure. The covalent and hydrogen bonds stabilizing the helix have influence on the final shape of the fibres.



Scheme 1. General outline of MS analysis of polypeptides derived from collagen

It could be a key-case (essential), and in the reverse process, that means during the hydrolysis, the 'cutted-up' strips due to regular structure take up characteristic for themselves conformations. Probably they have groups equally and in well-defined number spread all over the structure that are accessible for protonation during the MS measurements (Scheme 1). Hence, the situation is different as compared to small oligopeptides in which – as it was mentioned above – the number of protons attached is usually dependent on the number of basic amino acids in their structures (in other words, is dependent on the mass of the molecule).

Thus, collagen probably is not only synthesized in different way. It is also breaking down in different way. The fixed structure of the chains after the hydrolysis is presumably forced by the conformation reasons.

Summary

It is not easy to answer what is the real reason of the discussed unified ionization. In this article we presented some experimental details that confirm this pattern. It was also shown, on the basis of literature reports, that multiple ionization of peptides (during the MS measurements) is possible. Attempts to rationalize this problem were undertaken. However, the above discussion does not lead to a final conclusion. Up till now, the question remains rather unresolved. We would like to turn attention of the readers on this observation and attract investigators to further studies. This observation contributed to development of a convenient method for analysis of polypeptides, produced in technology of collagen hydrolysis.

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News from the Companies

Dokończenie ze strony 146

BADANIA I ROZWÓJ

Bydgoski producent dronów stawia na działalność badawczo-rozwojową

SoftBlue, bydgoski producent dronów, wypracował po czterech kwartałach 2015 roku ponad 7 mln PLN przychodów ze sprzedaży. To blisko 40% wzrost w stosunku do analogicznego okresu 2014 r. Firma dużo inwestuje w rozwój produktów idziałalność badawczo-rozwojową. W IV kwartale 2015 spółka skoncentrowała swoją pracę nadSenSoftem – urządzeniem, które pomaga mierzyć warunki, w jakich transportowane są produkty wrażliwe na zmiany temperatury czy poziomu wilgotności. W konsekwencji SoftBlue podpisało w ubiegłym miesiącu umowę z kanadyjską firmą –Leenlife Pharma International Inc. z siedzibą w Vacouver, dzięki której SenSoft będzie dostępny na tamtejszym rynku. Spółka otrzymała na ten cel finansowanie z programu GoGlobal w kwocie 130 tys. PLN. Pozyskane środki przeznaczy na stworzenie strategii wejścia czujników transportu SenSoft na rynek niemiecki, francuski, duński, szwedzki, norweski i fiński oraz działania promocyjne. Obecnie SoftBlue prowadzi m.in. prace badawcze nad AirDronem – urządzeniem do wykrywania zanieczyszczeń środowiska i SoftHeartem – urządzeniem do badania serca w widmach niesłyszalnych. *(kk)*

(Komunikat prasowy SoftBlue, 16.02.2016)

Polscy przedsiębiorcy i naukowcy wspólnie opracują unikalne w skali globalnej rozwiązania

Narodowe Centrum Badań i Rozwoju ogłosiło wyniki pierwszego konkursu „Projekty aplikacyjne". Konsorcja złożone z jednostek naukowych i przedsiębiorstw otrzymają na swoje nowatorskie projekty ponad 70 mln PLN. Konkurs w ramach poddziałania 4.1.4 „Projekty aplikacyjne" Programu Operacyjnego Inteligentny Rozwój był organizowany po raz pierwszy. Celem działania jest wsparcie najlepszych projektów B+R, obejmujących badania przemysłowe i eksperymentalne prace rozwojowe lub eksperymentalne prace rozwojowe, prowadzących do powstania polskich, unikalnych w skali globalnej rozwiązań. Zgłaszane w konkursie projekty, poza innowacyjnością w skali światowej, musiały się wpisywać w tzw. „Krajową inteligentną specjalizację". Premiowane były przedsięwzięcia o charakterze ponadregionalnym, a jednym z kryteriów oceny była opłacalność wdrożenia.

Zwycięskie konsorcja będą pracowały m.in. nad technologią produkcji elementów do zespołów napędowych przenośników przeznaczonych do pracy w ekstremalnych warunkach eksploatacyjnych, systemem do transportu gazu ziemnego, węglowodorów ciekłych oraz innych płynów pod średnim i wysokim ciśnieniem do zastosowania w przemyśle naftowym i gazowniczym, rozwiązaniami materiałowymi i konstrukcyjnymi i technologią produkcji niskostratnych przewodów napowietrznych, których celem jest ograniczenie strat przesyłowych w elektroenergetycznych liniach dystrybucyjnych oraz szeregiem rozwiązań z zakresu inżynierii materiałowej. *(kk)*

(http://www.nauka.gov.pl/, 22.02.2016)

KONKURSY, STYPENDIA, STAŻE

Ponad 4 mln PLN na powroty do badań naukowych

Łącznie ponad 4 mln PLN czeka na osoby z doktoratem, które chcą wrócić do prowadzenia badań po przerwie związanej z pracą w innym sektorze gospodarki lub z rodzicielstwem. Pierwszy konkurs w programie POWROTY organizuje Fundacja na rzecz Nauki Polskiej (FNP). Wnioski można składać do 15 kwietnia br. Konkurs POWROTY jest finansowany z POIR. Dofinansowanie mogą otrzymać projekty, których przedmiotem będą badania badawczo-rozwojowe, wpisujące się w zakres Krajowych Inteligentnych Specjalizacji. Szanse mają zwłaszcza te projekty, które dotyczą rozwoju technologii, zaprojektowania produktu czy procesu produkcyjnego i posiadają potencjał aplikacyjny lub znaczenie dla rozwiązania istotnego problemu naukowego czy społeczno-gospodarczego. Na 2-letni projekt można zdobyć 800 tys. PLN i przeznaczyć je m.in. na wynagrodzenia, prace zlecone, stypendia, szkolenia i staże w ramach rozwoju kadr oraz na pokrycie szeregu pozostałych kosztów związanych z prowadzeniem prac B+R. Ze wsparcia będą mogły skorzystać także firmy prowadzące działalność w Polsce. Mogą one otrzymać nawet do 80% dofinansowania na projekty w oparciu o zasady przyznawania pomocy publicznej. *(kk)*

(http://naukawpolsce.pap.pl/, 1.03.2016)

Dokończenie na stronie 153