PHYSICAL CHEMISTRY 2012

11th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Under the auspices of the
University of Belgrade

Proceedings

The Conference is dedicated to
Professor Ivan Draganić

September 24-28, 2012
Belgrade, Serbia

ISBN 978-86-82475-27-9 ***Volume*** **1**
ISBN 978-86-82475-28-6 ***Volume*** **II**

Title: PHYSICAL CHEMISTRY 2012 (Proceedings)
Editors: S. Anić and Ž. Čupić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 497; **Format**: B5; Printing finished in September 2012.

Text and Layout: "Jovan"

200- *Coppy printing*

CONTENTS

F-19-P

ANALYSIS OF THERMAL DENATURATION OF PEPSIN ON BASIS OF MALDI-TOF MS AND PAGE EXPERIMENTAL DATA

V. Pavelkić[1], K. Antić[1], M. Babić[1], K. Gopčević[2], M. Petkovic[3]

[1]Institute "Kirilo Savić", Vojvode Stepe 51, Belgrade, Serbia;
[2]School of Medicine, University of Belgrade,Višegradska 26, Belgrade, Serbia;
[3]Vinca Institute for Nuclear Sciences, POBox 522, Belgrade, Serbia

Abstract
To obtain detailed information about properties of pepsin in thermal denaturing conditions, polyacrylamide gel electrophoresis (PAGE) and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) experimental data were analyzed. These methods were used to analyze the changes in the structural properties of pepsin molecule subjected to broad-range temperature variations, from 25 ºC to 70 ºC, and pH range from 1 to 4.

Introduction
Porcine pepsin A (EC 3.4.23.1) belongs to the aspartic proteases. It is composed of two similar domains and is characterized by having two catalytic acid residues in the binding site [1]. It was shown that thermal modification of pepsin (followed by calorimetric studies) is complex process. It passes through two stages at different temperatures [2]. The aim of this work was to investigate influence of temperature and pH on porcine pepsin stability in a view of MALDI-TOF MS and PAGE methods.

Experimental
MALDI-TOF mass spectra were acquired on a Voyager Biospectrometry DE Pro Workstation (Perseptive Biosystems, Flamingham, MA, USA). The system utilises 20 Hz pulsed nitrogen laser emitting at 337 nm. The spectra were acquired in two mass ranges: from 500 Da to 10 kDa and from 10 kDa to 50kDa in the linear mode and under delayed extraction conditions. Low mass gate was switched on (at 450 Da and at 3500 Da, respectively). All spectra represent the average of 400 single laser shots.

Result and discussion
There are several peaks detectable in the high mass range (10 – 50 kDa) of the positive ion MALDI-TOF mass spectra of pepsin: at *m/z* about 34 kDa, which corresponds to the single positively charged ion of the protein, and at *m/z* about 17 000 which arises from the double-charged molecule. On the other hand, a series of peaks are detectable in the low mass range (500 Da to 10 kDa). The intensity and number of these peaks differs in dependence on the experimental conditions, *i.e.* pH and temperature applied. In Figure 1, MALDI-TOF mass spectra of pepsin incubated at pH (1- 4) and at various temperatures (from 25 ºC to 70 ºC) are shown. Two peaks arising from pepsin are

detectable in the left upside panel: at m/z=34 kDa and at m/z=17 kDa, which corresponds to the single- and double-positively charged molecule.

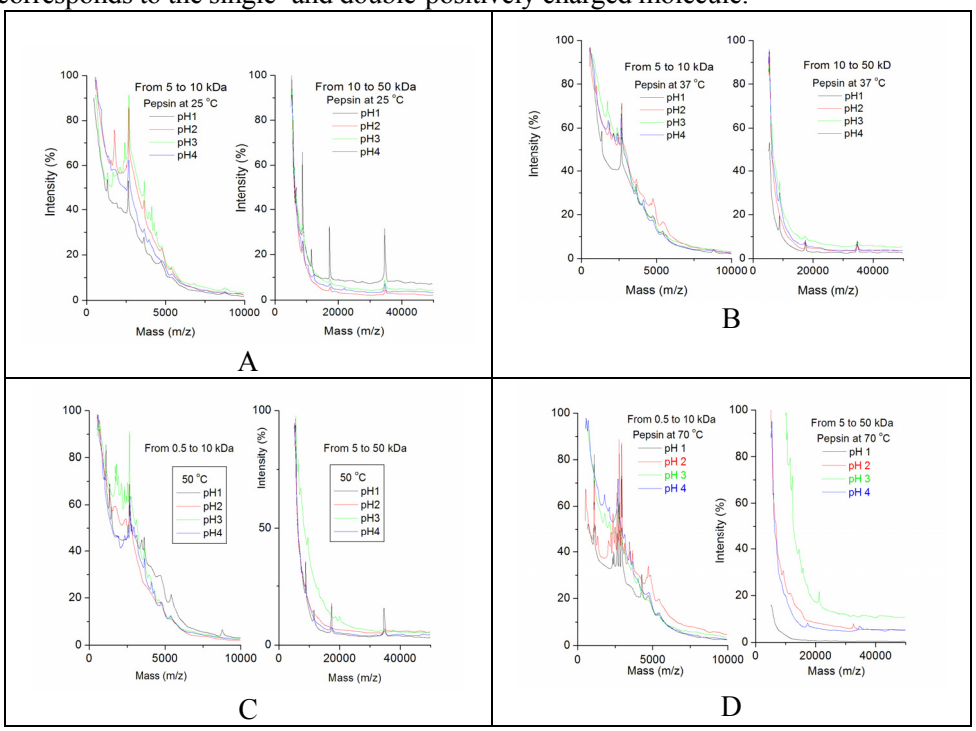

Figure 1. MALDI-TOF mass spectra of pepsin incubated at pH 1, 2, 3, and 4, at 25 ºC, 37 ºC, 50 ºC and 70º C (A, B, C, D respectively). The mass range from 0.5 to 10 kDa are presented at the left side of each panel A, B, C and D, whereas on the right, are presented the spectra acquired in the mass range from 10 to 50 kDa.

The intensity of these peaks depends on the temperature: at the lowest temperature applied, these peaks are the most intense, whereas at 70 ºC they are neglected. Small difference can be observed between the samples incubated at 37 ºC and 50 ºC (Fig 1B and 1C). In the low mass range, there are a number of peaks that most probably represent the peptide-degradation products of pepsin. The most intense signals arise from the group of peaks with similar masses that appear between *m/z*= 2500 and *m/z*=3000. The higher number of peaks is, detectable in the spectra of the sample incubated at 70 ºC, that implies the highest number of peptides that arise from pepsin in comparison to samples incubated at lower temperatures. With increase in the temperature the intensity of peaks arising from the native molecule decrease, along with increase in the number of peptides that arise from the molecule. There are, differences observed at pH 3 in comparison to pH1 and 2: the number of peaks arising from peptides in the low mass range is lower and the peaks that correspond to the native molecule disappear already at 50 ºC. The lowest number of peaks in the low mass region along with the rather low intensity of these peaks is detected in the spectra of pepsin incubated at pH 4 and at various temperatures. The differences in the intensities of the peaks that arise from the native molecule (higher mass range) are not

so pronounced like when the samples were incubated at lower pH values: the two peaks that correspond to the protein could be clearly observed even at the highest applied temperature.

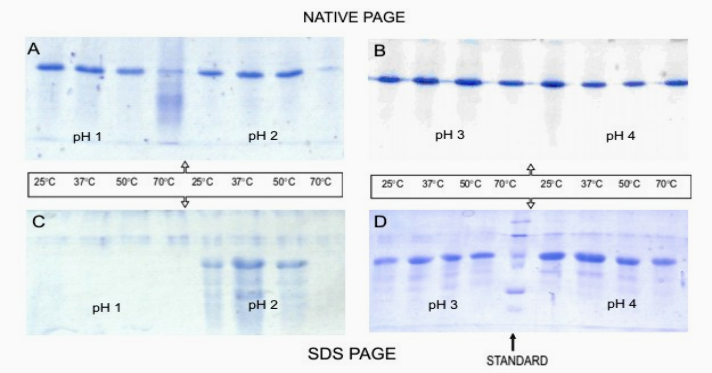

Figure 2. Native (A and B) and PAGE (C and D) electrophoregrams of pepsin on polyacrylamide gel according to the Laemmli procedure [3].

Native PAGE electrophoregrams show pepsin band at Rs value (distance of protein migration *vs.* distance of tracing dye migration) that corresponds to its native state at all investigated pH and temperature, except at pH 1 and 70°C, which yields 3 additional bands due to denaturation of the protein. On SDS-PAGE electrophoregrams (Figure 2C and 2D), there is an absence of protein band at pH 1, which may be due to the achievements of iso-electric point of pepsin. At pH 2, 3, and 4 (Figure 1D) besides the main pepsin band, there are numbers of bands that represent the fragments with smaller molecular masses – degradation products of denaturized pepsin. Calculated molecular weights of those fragments are in the range from 18 – 28 kDa.

Conclusion

These results imply that the protein is most stable at pH 4. There is an inverse relationship between the presence and intensity of the peaks in the higher mass range and in the lower range obtained from MALDI-TOF MS experiments. The results obtained by two methods are in good agreement.

Acknowledgement

This study was supported by Ministry of Education and Science of Republic of Serbia, Project OI 172015.

References

[1] T. Tanaka, R. Y. Yada. Biochem J., (1996), 315, 443-446.
[2] V. M. Pavelkic, M. V Beljanski, K. M. Antic, M. M. Babic, T. P. Brdaric, K .R. Gopcevic, RJPC (2011), 85, 2245-2250.
[3] Laemmli, Nature, 1970, 227, 680-685.

CIP Volume I

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

544(082)
621.35(082)
66.017/.018(082)

MEĐUNARODNA konferencija iz fundamentalne i primenjene fizičke hemije (11 ; 2012 ; Beograd)
Physical Chemistry 2012 : proceedings. #Vol. #1 / 11th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 24-28, 2012, Belgrade ; [editors S.[Slobodan] Anić and Ž.[Željko] Čupić ; organized by Society of Physical Chemists of Serbia ... et al.]. - Belgrade : Society of Physical Chemists of Serbia, 2012 (Belgrade : Jovan). - VI, 498 str. : ilustr. ; 24 cm

"The Conference is dedicated to Professor Ivan Draganić" --> nasl. str. - Tiraž 200. - Bibliografija uz svaki rad.

ISBN 978-86-82475-27-9
1. Društvo fizikohemičara Srbije (Beograd)
a) Физичка хемија - Зборници b) Електрохемијско инжењерство - Зборници c) Наука о материјалима - Зборници
COBISS.SR-ID 193432332