PHYSICAL CHEMISTRY 2006

Proceedings

of the 8th International Conference on Fundamental and Applied Aspects of Physical Chemistry

September 26-29,
Belgrade, Serbia

TEMPERATURE AND Al^{3+} INFLUENCE ON ELECTROPHORETIC MOBILITY OF PORCINE PEPSIN

V. Pavelkić[1], K. Gopčević[2], D. Krstić[2] and M. Ilić[3]

[1] *Vinca-Institute of Nuclear Science, Department of Physical Chemistry, P.O.Box 522, Belgrade*
[2] *School of Medicine, University of Belgrade, Višegradska 26, Belgrade,*
[3] *Institute of General and Physical Chemistry, Studentski trg 12-16, Belgrade, Serbia*

Abstract

The influence of temperature and different concentrations of Al^{3+} on pepsin electrophoretic mobility was investigated. The increase of Al^{3+} concentrations causes the decrease the electrophoretic mobility of enzyme. Also the increase of temperature induced the same effect. The influence of both temperature and Al^{3+} ion concentrations is additive.

Introduction

Pepsin, an acidic protease, is the principal proteolytic enzyme of gastric juice. The primary and tertiary structures of pepsin are highly homologous to other aspartatic proteases. Porcine pepsin was the first aspartic protease to have its complete amino acid sequence determined [1]. The molecular weight is 35000 Da. The protein consists of 326 residues. The structure is bilobal, consisting of two predominantly β-sheet lobes related by a pseudo 2-folded axis [2, 3].

It is known that temperature and presence of Al^{3+} ion influence the three dimensional structure of proteins, as well as its net charge. Thus, the electrophoretic mobility of proteins is affected [4]. That influence depends on concentration of presenting ion and temperature range. The aim of this work was to examine the influence of Al^{3+} ion (in the concentration range from 1 – 10 mM) and temperature (from 25 – 70 °C), on electrophoretic moiety and enzyme activity of pepsin.
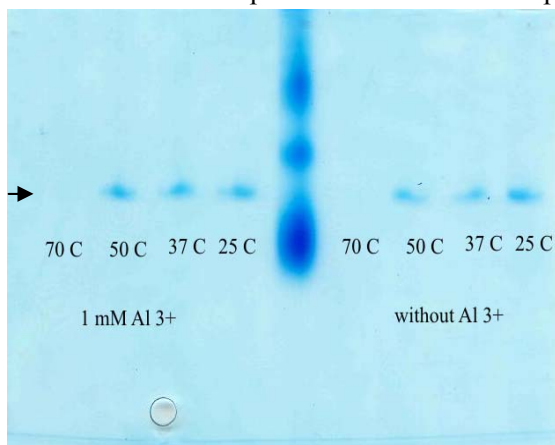
Experimental

Pepsin was purchased from Sigma Chemical Co. and used without further purification. Other chemicals were of reagent grade and were prepared prior to use. Water solutions of all samples (2 mg/mL of pepsin were dissolved in water) were titrated with HCl to pH 2, with addition of different concentrations of Al^{3+} ion (1, 5 and 10 mM; AlCl$_3$ x 6H$_2$O as a source of Al^{3+} ion was used). The samples were heated and thermostated for 10 minutes to obtain desired temperature (25, 37, 50 and 70 °C). Native electrophoresis of pepsin on polyacrylamide gel was carried out at 4°C according to the Laemmli procedure [5]. Electrophoresis was done at 4 °C during 90 min. Visualization was performed with Commassie Brilliant Blue G-250 dye. The gels are scanned and processed using Corel Drow.10.0 software package. Quantification of electrophoretic mobility of the molecule is carried out via R$_f$ value, where it is defined by:

R$_f$ = [distance of protein migration] / [distance of tracing dye migration]

Results and Disscusion

The electrophoregrams of pepsin samples treated at different temperature values in absence or in the presence of Al^{3+} ion are presented in Figures 1 and 2. It could be seen that in all cases increasing the temperature causes the decrease in electrophoretic moiety of pepsin. The decrease in electrophoretic moiety can be explained by thermally induced conformational changes in pepsin molecule. However, the pepsin bend is absent in samples treated at 70 °C, in the presence of all investigated Al^{3+} concentrations, as well as in the absence of Al^3 ion. This result is in agreement with previously reported data that temperatures of 70 °C and higher induce denaturation of an enzyme [6]. Moreover, the degree of pepsin electrophoretic mobility decrease depends on Al^{3+} concentration which the one has been exposed. For example, difference between Rs values obtained at 25 °C and 50 °C in absence of Al^{3+} ion is 0.02, while in the presence of 10 mM Al^{3+} it is 0.05 (Table 1.). If we discuss the influence of Al^{3+} ion concentration on pepsin mobility at defined temperature it could be seen that increase in concentration of Al^{3+} decelerate the migration of pepsin samples on concentration dependent manner.(Table 1). For example, Rs values of pepsin at 37 °C in the absence of Al^{3+} is 0.47,

Fig. 1. Native PAGE electrophoregram of pepsin at 25 °C, 37 °C , 50 °C and 70 °C, without Al^{3+} and in a presence of 1 mM Al^{3+} at pH 2.
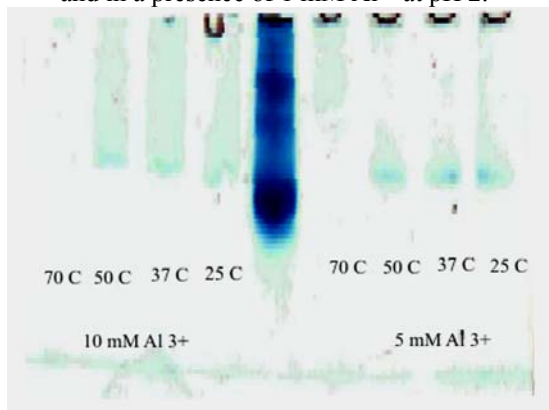
Fig. 2. Native PAGE electrophoregram of pepsin at 25 °C, 37 °C, 50 °C and 70 °C, in a presence of 5mM and 10 mM Al^{3+} at pH 2.

while Rs values are 0.46, 0.44and 0,42 in the presence 1mM, 5mM and 10mM of Al^{3+}, respectively. The same trend was obtained for the other tested temperatures, except for 70 °C. The slow down in pepsin migration can be explained by conformational changes caused by Al^{3+} binding to enzyme. It is obviously that the highest investigated temperature and highest concentration of Al^{3+} result in the lowest electrophoretic mobility, and that influence is additive.

Table 1. R_f values of pepsin treated at different temperatures without and with various Al^{3+} ions concentrations

C_{Al}^{3+} (mM)	R_s (25 °C)	R_s (37°C)	R_s (50°C)	R_s (70°C)
0	0.47	0.47	0.45	0
1	0.47	0.46	0.45	0
5	0.45	0.44	0.44	0
10	0.44	0.42	0.39	0

Conclusion

In the absence of Al^{3+} at all tested temperatures the electrophoretic mobility is the highest. Pepsin samples treated with different concentrations of Al^{3+} ions at pH 2 show the difference in electrophoretic mobility: increase in concentration caused decrease in Rs values. At the same time, increase in temperature cause the same effect – decrease in electrophoretic mobility.

References:

[1] J. Tang, R. N. S. Wang, J. Cell Biochem, 1987, **33**, 53-63.
[2] R. Sielecki, A. A. Fedorov, A. Boodhoo, N. S. Andreeva, M. N. G. James, J.Mol. Biol., 1990, **24**, 143-170.
[3] J. B.Cooper, G. Khan, G. Taylor, I. J. Tickle, T. L. Blundell, J. Mol. Biol., 1990, **214**, 199-222.
[4] A. Dorney et al., J. Inorg. Biochem., 2003, **97**, 118-123.
[5] Laemmli, Nature, 1970, **227,** 680-685.
[6] Privalov, P.L., Mateo, P.L., and Khechinashvili, N.N., J. Mol.Biol., 1981, **152**, 445-464.