PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

September 21-23, 2004
Belgrade, Serbia and Montenegro

PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

Editors
A. Antić-Jovanović and S. Anić

ISBN	86-82457-12-x
Title:	Physical Chemistry 2004. (Proceedings)
Editors	A. Antić-Jovanović and S. Anić
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 137, 11001 Belgrade, Serbia and Montenegro
Publisher:	Society of Physical Chemists of Serbia
Printed by:	"Jovan" Printing and Published Comp; 300 Copies; Number of Pages: x + 906; Format B5; Printing finished in September 2004.
Text and Layout:	Aleksandar Nikolić

300 – copy printing

KINETICS OF HEAT DENATURATION OF PEPSIN IN A STRONG ACID MEDIA

V. Pavelkić[1], M. Živanović[1], V. Spasojević – Tišma[1] and M. Ilić[2]
[1]*Vinča Institute of Nuclear Sciences, Department of Physical Chemostry, POBox 522, 11001 Belgrade,*
[2]*Institute of General and Physical Chemistry, Studentski trg 12-16, 11000 Belgrade*

Abstract

Heat aggregation of pepsin, in a strong acid media; involve the stage of nucleation, and the stage of growth of aggregates. The initial parts of the kinetic curves of aggregation were followed via monitoring the increase of absorbance (A) and were linearized as {dA/dt; t} and {A; t^2} functions. The slope of these curves is proportional to the product rate constant of reversible denaturation and the rate constant of growth of aggregates. Addition of Al^{3+} ions display a lag period whose appearance is caused by intramolecular predenaturational changes in the pepsin molecule.

Introduction

Heat denaturation of proteins is often accompanied by aggregation of denatured protein molecules [1]. Accumulation of aggregates may be easily registrated via monitoring the increase of absorbance. According the theory proposed by Patro and Przybycien [2], the initial stage of reversible protein aggregation involves the reaction of nucleation and the reaction of growth of aggregates. Mechanism can be shematically presented as:

$$N \xrightarrow{k_{den}} D \qquad (1)$$

$$nD \leftrightarrow D_n \text{ (nucleus)} \qquad (2)$$

$$D_n + D \xrightarrow{k_{agg}} D_{n+1} \qquad (3)$$

where: N and D are the native and denatured states of a protein, respectively.
k_{den} – is the rate constant of denaturation.
N – is the number of ″monomer″ D participating in the formation of the nucleus.
k_{agg} – is the rate constant of the stage of growth of aggregates.

In this paper we presented the kinetic parameters that follow heat aggregation of pepsin in a strong acid media (pH 2), at 37 °C , and the influence of Al^{3+} on kinetic agregation of pepsin at the same experimental condition.

Experimental

Porcine pepsin was purshesed from Sigma Chem. Co. and used without further purification. Other chemicals were of reagent grade and were prepared prior to use.

UV absorbance measurements were carried out Perkin Elmer, Lambda 35 UV-VIS spectrophotometer with temperature control cell units. The change in absorbance was measured at 280 nm and 37 °C.

Results and Discussion

The kinetic curve of heat aggregation of pepsin at 37 °C and pH 2 is presented in Figure 1A. At sufficiently low values of time (t) the accumulation of denatured form D proceeds linearly in time: $[D] = k_{den} [N_0] t$, where $[N_0]$ is initial concentration of pepsin (0.015 mM). For initial parts of the kinetic curves registraded value of absorbance A is proportional to the amount of aggregates formed and the rate of the change in the A value, dA_{280} / dt is proportional to the product $k_{agg} [D]$ (Figure 1B.). Acording to Patro and Przybycien theory [2], and taking into account the linear character of accumulation of the form D, change dA/dt will be proportional to $k_{agg} k_{den} [N_0] t$. Integration of this equation shows that at sufficiently low values of time value A become proportional to t^2 (Figure 1C.).

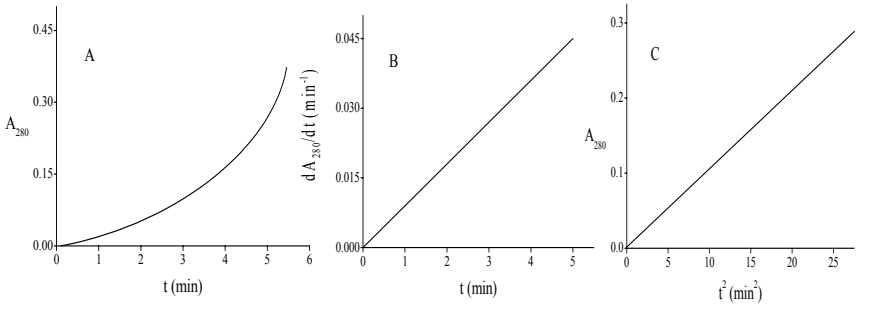

Figure1. Heat aggregation of pepsin: A.Time dependece of the increase in absorbance of pepsin at 280 nm (pH 2; 37 °C); B and C linearisation in the coordinate $\{dA_{280} / dt; t\}$ and $\{A_{280} ; t^2\}$, respectively .

The assumption was that the nuclei concentration remain constant. Calculated values of k_{den} and k_{agg} for pepsin at pH 2 were (0.0092 ± 0.0004) min^{-1} and (0.0101 ± 0.0004) min^{-1} respectively.

Addition of Al^{3+} (0.015 mM) causes a delayed character of the kinetic curve. The appeatrence of a lag perion on the dA / dt versus t curve maens that the formation of the denatured form is proceded by the predenaturational changes in the pepsin molecule [3]. The slope of the linear part of the (dA / dt) = f (t) curve affter passing a lag period is proportional to the rate of accumulation of the D form (Figure 2 A nad B).

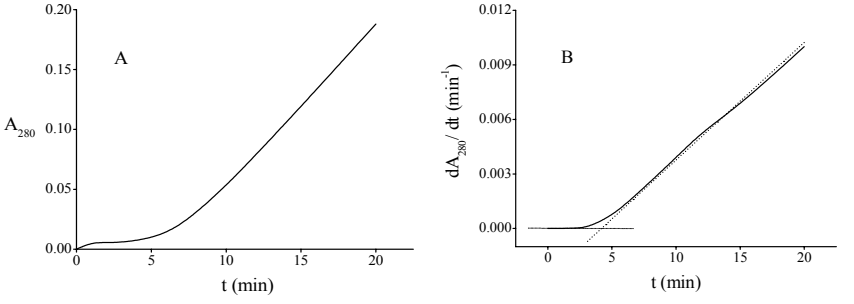

Figure 2. Heat aggregation of pepsin in a presence of Al^{3+} ions; A. Dependence of absorbance at 280 nm on time; B. Linearisation in coordinates {dA / dt; t} at [Pepsin] = 0.015 mM, and [Al^{3+}] = 0.015 mM.

Calculated value of k_{den} of pepsin in a presence of Al^{3+} ions, after 5 min of lag period is (0.00068 ± 0.00005), i.e. bound Al^{3+} on pepsin slow down the aggregation and lead to stabilization of pepsin molecule.

Conclusion

Heat aggregation of pepsin, in a strong acid media involves the stage of nucleation, and the stage of growth of aggregates. Addition of Al^{3+} causes a delayed character of the kinetic curve and also slow down the aggregation and lead to stabilization of pepsin molecule.

Acknowledgement

Financial support was provided by the MSTD of the Republic of Serbia, Proj. No. 1991.

References

[1] B.A. Kornialev, B.I. Kurganov, T.B. Eronina, N.A. Chebotareva, N.B. Livanova, V.N. Orlov, V.Y. Chernyak, Mol. Biol. (Moscow), 1997, 31, 82.
[2] S.Y. Patro, T.M. Przybycien, Biophysical Journal, 1996, 2888.
[3] U. Jakob, H. Lilie, I. Meyer, J. Buchner, J.Biol.Chem., 1995, 270, 7288.