

Interaction enthalpies of α -chymotrypsin with water-acetone mixtures

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Abstract

© 2017 by Nova Science Publishers, Inc. All rights reserved. The aim of our investigation is to provide a thermochemical description of the stabilizing/destabilizing effects of organic solvents on the enzyme stability to elucidate what intermolecular processes produce the main effect on the stability and functions of the enzymes at low, intermediate, and high water content in organic liquids. Bovine pancreatic A-chymotrypsin was utilized as a model enzyme. Isothermal calorimetry measurements were performed to study the stability of A-chymotrypsin in water-acetone mixtures. Interaction enthalpies of A-chymotrypsin with water-acetone mixtures have been measured at 25°C. Our results clearly show that isothermal calorimetry is an effective experimental tool for studying the simultaneous action of water and organic liquids on the stability of enzyme macromolecules. Thermochemical results were compared with the enzyme activity data. The degree of stabilization/destabilization of achymotrypsin depends significantly on the water content in acetone. There are three concentration regimes. At high water content, the residual enzyme activity values are close to 100%. At intermediate water content, residual enzyme activity is close to zero. The residual catalytic activity in the water-poor acetone is ~80%.

References

- [1] Gregory, R. B. (1995). Protein hydration and glass transition behavior. In: Protein-Solvent Interactions (Gregory, R. B., ed.), Marcel Dekker, New York, 191-264.
- [2] Rupley, J. A., Careri, G. (1991). Protein hydration and function. *Adv. Protein Chem.* 41, 37-172.
- [3] Kuntz, I. D., Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Adv. Protein Chem.* 28, 239-345.
- [4] Oleinikova, A., Smolin, N., Brovchenko, I., Geiger, A., Winter, R. (2005). Properties of spanning water networks at protein surfaces. *J. Phys. Chem. B.* 109, 1988-1998.
- [5] Durchschlag, H., Zipper, P. (2001). Comparative investigations of biopolymer hydration by physicochemical and modeling techniques. *Biophys. Chem.* 93, 141-157.
- [6] Sirotkin, V. A., Khadiullina, A. V. (2013). Gibbs energies, enthalpies, and entropies of water and lysozyme at the inner edge of excess hydration. *J. Chem. Phys.* 139, 075102/1-075102/9.
- [7] Sirotkin, V. A., Khadiullina, A. V. (2014). A study of the hydration of ribonuclease A using densitometry: Effect of the protein hydrophobicity and polarity. *Chem. Phys. Lett.* 603, 13-17.
- [8] Privalov, P. L., Crane Robinson, C. (2017). Role of water in the formation of macromolecular structures. *Eur. Biophys. J.* 46, 203-224.
- [9] Kijima, T., Yamamoto, S., Kise, H. (1996). Study of tryptophan fluorescence and catalytic activity of α -chymotrypsin in aqueous organic media. *Enz. Microb. Technol.* 18, 2-6.
- [10] Sirotkin, V. A., Hütt, R., Wolf, G. (2005). Thermochemical investigations of hydrolysis of p-nitrophenyl acetate in wateracetone mixtures, *Thermochim. Acta* 426, 1-6.

- [11] Khmel'nitsky, Yu., Mozhaev, V. V., Belova, A. B., Sergeeva, M. V., Martinek, K. (1991). Denaturation capacity: A new quantitative criterion for selection of organic solvents as reaction media in biocatalysis. *Eur. J. Biochem.* 198, 31-41.
- [12] Partridge, J., Moore, B. D., Halling, P. J. (1999). A-Chymotrypsin stability in aqueous-acetonitrile mixtures: Is the native enzyme thermodynamically or kinetically stable under low water conditions? *J. Mol. Cat. B: Enzym.* 6, 11-20.
- [13] Griebenow, K.; Klibanov, A. M. (1996). On protein denaturation in aqueous-organic mixtures but in pure organic solvents. *J. Amer. Chem. Soc.* 118, 11695-11700.
- [14] Klibanov, A. M. (2001). Improving enzymes by using them in organic solvents, *Nature* 409, 241-246.
- [15] Halling, P. J. (2004). What can we learn by studying enzymes in nonaqueous media? *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1287-1297.
- [16] Lousa, D., Baptista, A. M., Soares, C. M. (2013). A molecular perspective on nonaqueous biocatalysis: Contributions from simulation studies. *Phys. Chem. Chem. Phys.* 15, 13723-13736.
- [17] Carrea, G., Riva, S. (2000). Properties and synthetic applications of enzymes in organic solvents, *Angew. Chem. Int. Ed.* 39, 2226-2254.
- [18] Micaelo, N. M., Soares, C. M. (2007). Modeling hydration mechanisms of enzymes in nonpolar and polar organic solvents, *FEBS J.* 274, 2424-2436.
- [19] Clark, D. S. (2004). Characteristics of nearly dry enzymes in organic solvents: implications for biocatalysis in the absence of water, *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 359, 1299-1307.
- [20] Serdakowski, A. L., Dordick, J. S. (2007). Enzyme activation for organic solvents made easy, *Trends Biotechnol.* 26, 54-48.
- [21] Rariy, R., Klibanov, A. M. (1997). Correct protein folding in glycerol, *Proc. Natl. Acad. Sci. U.S.A.* 94, 13520-13523.
- [22] Bull, H. B. (1944). Adsorption of water vapor by proteins. *J. Amer. Chem. Soc.* 66, 1499-1507.
- [23] Luscher-Mattli, M., Ruegg, M. (1982). Thermodynamic functions of biopolymer hydration. I. Their determination by vapor pressure studies, discussed in an analysis of the primary hydration process. *Biopolymers* 21, 403-418.
- [24] Luscher-Mattli, M., Ruegg, M. (1982). Thermodynamic functions of biopolymer hydration. II. Enthalpy-entropy compensation in hydrophilic hydration process. *Biopolymers* 21, 419-429.
- [25] Bone, S. (1987). Time-domain reflectometry studies of water binding and structural flexibility in chymotrypsin. *Biochem. Biophys. Acta.* 916, 128-134.
- [26] Hnojewyj, W. S., Reyerson, L. H. (1961). Further studies on the sorption of H₂O and D₂O vapors by lysozyme and the deuteriumhydrogen exchange effect. *J. Phys. Chem.* 65, 1694-1698.
- [27] Sirotkin V. A., Korolev D. V. (2005). Effect of acetonitrile on the hydration of human serum albumin films: a calorimetric and spectroscopic study. *Thermochim. Acta*, 432, 246-253.
- [28] Sirotkin, V. A., Khadiullina, A. V. (2011). Hydration of proteins: excess partial enthalpies of water and proteins. *J. Phys. Chem. B*, 115, 15110-15118.
- [29] Sirotkin, V. A., Komissarov, I. A., Khadiullina, A. V. (2012). Hydration of proteins: excess partial volumes of water and proteins, *J. Phys. Chem. B*, 116, 4098-4105.
- [30] Borisover, M. D., Sirotkin, V. A., Solomonov, B. N. (1995). Isotherm of water sorption by human serum albumin in dioxane: Comparison with calorimetric data. *J. Phys. Org. Chem.* 8, 84-88.
- [31] Sirotkin, V. A., Borisover, M. D., Solomonov, B. N. (1995). Heat effects and water sorption by human serum albumin on its suspension in water-dimethyl sulfoxide mixtures. *Thermochim. Acta*, 256, 175-183.
- [32] Sirotkin, V. A. (2005). Effect of dioxane on the structure and hydration-dehydration of A-chymotrypsin as measured by FTIR spectroscopy. *Biochim. Biophys. Acta*, 1750, 17-29.
- [33] Kocherbitov, V., Arnebrant, T. (2010). Hydration of Lysozyme: The Protein-Protein Interface and the Enthalpy-Entropy Compensation. *Langmuir*, 26, 3918-3922.
- [34] Foss, J. G., Reyerson, L. H. (1958). The sorption of water vapor by lyophilized ribonuclease. *J. Phys. Chem.* 62, 1214-1216.
- [35] Amberg C. H. (1957). Heats of adsorption of water vapor on bovine serum albumin. *J. Amer. Chem. Soc.* 79, 3980-3984.
- [36] Almog R., Schrier E. E. (1978). The dependence of the heat of solution of lyophilized solid ribonuclease A on water content. *J. Phys. Chem.* 82, 1701-1702.
- [37] Smith A. L., Shirazi H. M., Mulligan S. R. (2002). Water sorption isotherms and enthalpies by lysozyme using the quartz crystal microbalance/heat conduction calorimeter. *Biochim. Biophys. Acta*, 1594, 150-159.
- [38] Kocherbitov V., Arnebrant T., Söderman O. (2004). Lysozyme-water interactions studied by sorption calorimetry. *J. Phys. Chem. B*, 108, 19036-19042.

- [39] Sirotkin, V. A., Zinatullin, A. N. Solomonov, B. N., Faizullin, D. A., Fedotov, V. D., (2002). Interaction enthalpies of solid bovine pancreatic chymotrypsin with organic solvents: comparison with FTIR-spectroscopic data. *Thermochim. Acta*, 382, 151-160.
- [40] Fersht, A. (1999). *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*; Freeman & Co: New York.
- [41] Lehninger, A. L.; Nelson, D. L.; Cox, M. M. (1993). *Principles of Biochemistry*; Worth: New York.
- [42] Perrin, D. D., Armarego, W. L. F., Perrin, D. R. (1980). *Purification of Laboratory Chemicals*, Oxford: Pergamon Press.
- [43] Kogan, V. B., Fridman, B. N., Kafarov, V. V. (1966). *Liquid-Vapor Equilibrium*. Moscow: Nauka.
- [44] Bell, G., Janssen, A. E. M., Halling, P. (1996). Water activity fails to predict critical hydration level for enzyme activity in polar organic solvents: Interconversion of water concentrations and activities. *Enzym. Microb. Technol.* 20, 471-476.
- [45] Nikolskii, B. P. (1963). *Chemist's Handbook*, Leningrad, Russia: Goskhimizdat.
- [46] Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Bur. Stand., Sect. A* 81, 89.
- [47] Sirotkin, V. A., Kuchierskaya, A. A. (2017). α -Chymotrypsin in water-ethanol mixtures: Effect of preferential interactions. *Chem. Phys. Lett.* 689, 156-161.
- [48] Sirotkin, V. A., Kuchierskaya, A. A. (2017). α -Chymotrypsin in water-acetone and water-dimethyl sulfoxide mixtures: Effect of preferential solvation and hydration. *Proteins: Functions, Structure and Bioinformatics*, 85, 1808-1819.
- [49] Khurgin, Y. I., Sherman, F. B., Tusupkaliev, U. (1978). Temperature dependence of chymotrypsinogen A hydration in dynamic conditions. *Mol. Biol.* 12, 572-579.
- [50] Sirotkin, V. A., Kuchierskaya, A. A. (2017). Preferential solvation/hydration of α -chymotrypsin in water-acetonitrile mixtures. *J. Phys. Chem. B.* 121, 4422-4430.
- [51] Sirotkin, V. A., Kuchierskaya, A. A. (2017). Lysozyme in water-acetonitrile mixtures: Preferential solvation at the inner edge of excess hydration. *J. Chem. Phys.* 146, 215101-8.