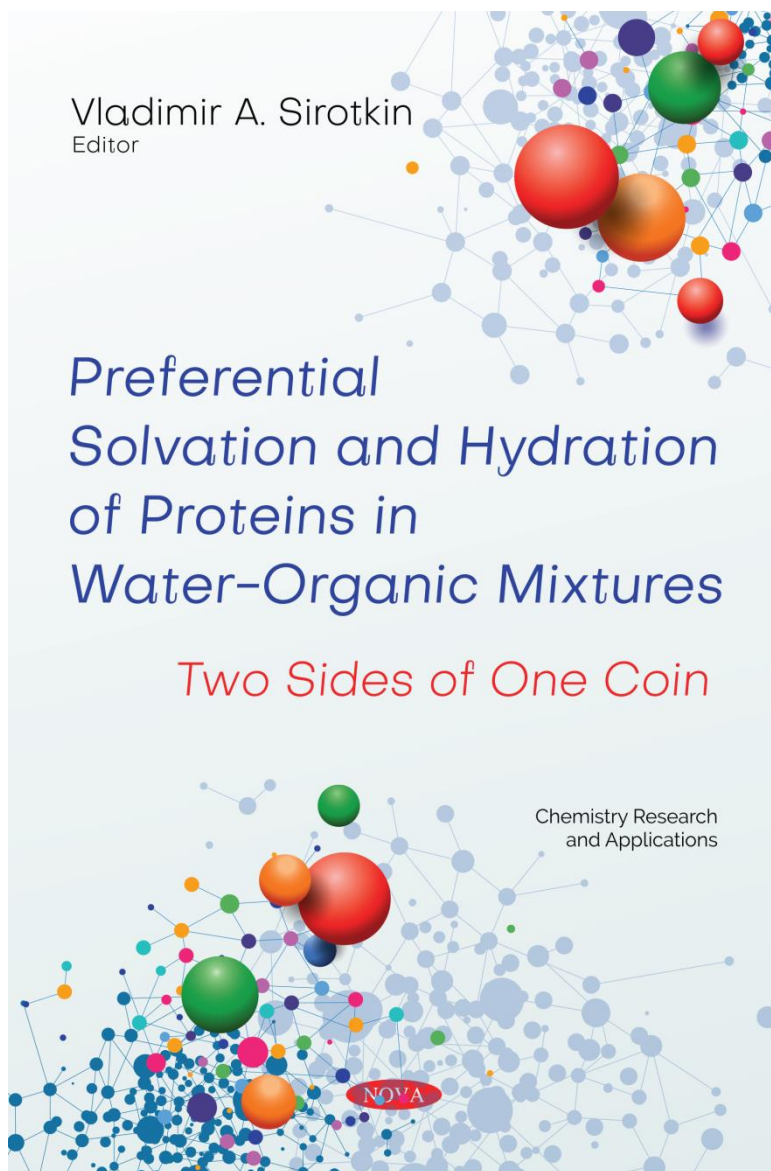


Sirotkin, V.A. Preferential solvation of lysozyme in water-dimethyl sulfoxide mixtures: Gibbs energies of water and organic solvent. / In: Sirotkin V.A., (Ed.) Preferential solvation and hydration of proteins in water-organic mixtures: Two sides of one coin. - Nova Science Publishers, Inc., Hauppauge, NY, 2019. - P.91-122.



Chapter 4

**PREFERENTIAL SOLVATION OF
Lysozyme IN WATER-DIMETHYL
SULFOXIDE MIXTURES: GIBBS ENERGIES OF
WATER AND ORGANIC SOLVENT**

Vladimir A. Sirotkin^{*}

A.M. Butlerov Institute of Chemistry, Kazan Federal University,
Kazan, Russia

ABSTRACT

Water/organic solvent sorption and residual enzyme activity were investigated to monitor preferential solvation and preferential hydration of protein macromolecules in the entire range of water content in organic liquids at 25°C. This approach was applied to estimate protein destabilization/stabilization due to the preferential interactions of hen-egg-white lysozyme with water-dimethyl sulfoxide (DMSO) mixtures. This approach facilitates the individual evaluation of the Gibbs energies of water, protein, and organic solvent. There are two concentration regimes. Lysozyme is preferentially hydrated at high water content. The residual enzyme activity values are close to 100%. At low water content, lysozyme has a higher affinity for DMSO than for water. The DMSO-induced irreversible inactivation of α -chymotrypsin was found in this region. Residual enzyme activity is close to zero in this concentration range.

Keywords: protein hydration, preferential solvation, enzyme activity, hen-egg-white lysozyme, dimethyl sulfoxide (DMSO)

^{*} Corresponding author: Vladimir A. Sirotkin, Kazan Federal University, A.M. Butlerov Institute of Chemistry, Kremlevskaya str., 18, Kazan, 420008 Russia, Email: vsir@mail.ru.

REFERENCES

- [1] Timasheff, S. N. (2002). Protein-solvent preferential interactions, protein hydration, and the modulation of biochemical reactions by solvent components. *Proc. Natl. Acad. Sci. USA.*, *99*, 9721-9726.
- [2] Arakawa, T., Kita, Y. & Timasheff, S. N. (2007). Protein precipitation and denaturation by dimethyl sulfoxide. *Biophys. Chem.*, *131*, 62-70.
- [3] Gekko, K., Ohmae, E., Kameyama, K. & Takagi, T. (1998). Acetonitrile-protein interactions: Amino acid solubility and preferential solvation. *Biochim. Biophys. Acta*, *1387*, 195-205.
- [4] Kovrigin, E. L. & Potekhin, S. A. (2000). On stabilizing action of protein denaturants: Acetonitrile effect on stability of lysozyme in aqueous solutions. *Biophys. Chem.*, *83*, 45-59.
- [5] Shimizu, S. & Matubayasi, N. (2014). Preferential solvation: Dividing surface vs excess numbers. *J. Phys. Chem. B.*, *118*, 3922-3930.
- [6] Auton, M., Bolen, D. W. & Rosgen, J. (2008). Structural thermodynamics of protein preferential solvation: Osmolyte solvation of proteins, amino acids, and peptides. *Proteins*, *73*, 802-813.
- [7] Casassa, E. F. & Eisenberg, H. (1964). Thermodynamic analysis of multicomponent solutions. *Adv. Protein Chem.*, *19*, 287-395.
- [8] Tanford, C. (1969). Extension of theory of linked functions to incorporate effects of protein hydration. *J. Mol. Biol.*, *39*, 539-544.
- [9] Schellman, J. A. (1987). Selective binding and solvent denaturation. *Biopolymers*, *26*, 549-559.
- [10] Arakawa, T., Bhat, R. & Timasheff, S. N. (1990). Why preferential hydration does not always stabilize the native structure of globular proteins. *Biochem.*, *29*, 1924-1931.
- [11] Smith, P. E. (2006). Equilibrium dialysis data and the relationships between preferential interaction parameters in biological systems in

- terms of Kirkwood-Buff integrals. *J. Phys. Chem. B*, *110*, 2862–2868.
- [12] Parsegian, V. A., Rand, R. P. & Rau, D. C. (2000). Osmotic stress, crowding, preferential hydration, and binding: A comparison of perspectives. *Proc. Natl. Acad. Sci. U.S.A.*, *97*, 3987–3992.
- [13] Kirby Hade, E. P. & Tanford, C. (1967). Isopiestic compositions as a measure of preferential interactions of macromolecules in two-component solvents. Application to proteins in concentrated aqueous cesium chloride and guanidine hydrochloride. *J. Amer. Chem. Soc.*, *89*, 5034-5040.
- [14] Kamiyama, T., Liu, H. L. & Kimura, T. (2009). Preferential solvation of lysozyme by dimethyl sulfoxide in binary solutions of water and dimethyl sulfoxide. *J. Therm. Anal. Cal.*, *95*, 353-359.
- [15] Reisler, E., Haik, Y. & Eisenberg, H. (1977). Bovine serum albumin in aqueous guanidine hydrochloride solutions. Preferential and absolute interactions and comparison with other systems. *Biochem.*, *16*, 197-203.
- [16] Izumi, T., Yoshimura, Y. & Inoue, H. (1980). Solvation of lysozyme in water/dioxane mixtures studied in the frozen state by NMR spectroscopy. *Arch. Biophys. Biochem.*, *200*, 444-451.
- [17] Gregory, R. B. (1995). Protein hydration and glass transition behavior. In: *Protein-Solvent Interactions* (Gregory, R.B., ed.), Marcel Dekker, New York, 191-264.
- [18] Rupley, J. A. & Careri, G. (1991). Protein hydration and function. *Adv. Protein Chem.*, *41*, 37-172.
- [19] Kuntz, I. D. & Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Adv. Protein Chem.*, *28*, 239-345.
- [20] 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.
- [21] Durchschlag, H. & Zipper, P. (2001). Comparative investigations of biopolymer hydration by physicochemical and modeling techniques. *Biophys. Chem.*, *93*, 141-157.

- [22] 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.
- [23] 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.
- [24] Privalov, P. L. & Crane-Robinson, C. (2017). Role of water in the formation of macromolecular structures. *Eur. Biophys. J.*, *46*, 203-224.
- [25] Bull, H. B. (1944). Adsorption of water vapor by proteins. *J. Amer. Chem. Soc.*, *66*, 1499-1507.
- [26] 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.
- [27] Luscher-Mattli, M. & Ruegg, M. (1982). Thermodynamic functions of biopolymer hydration. II. Enthalpy-entropy compensation in hydrophilic hydration process. *Biopolymers*, *21*, 419-429.
- [28] Bone, S. (1987). Time-domain reflectometry studies of water binding and structural flexibility in chymotrypsin. *Biochem. Biophys. Acta.*, *916*, 128-134.
- [29] Sirotkin, V. A. & Khadiullina, A. V. (2011). Hydration of proteins: excess partial enthalpies of water and proteins. *J. Phys. Chem. B*, *115*, 15110-15118.
- [30] 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.
- [31] Sirotkin, V. A. (2005). Effect of dioxane on the structure and hydration-dehydration of α -chymotrypsin as measured by FTIR spectroscopy. *Biochim. Biophys. Acta.*, *1750*, 17-29.
- [32] Klibanov, A. M. (2001). Improving enzymes by using them in organic solvents, *Nature*, *409*, 241-246.
- [33] Carrea, G. & Riva, S. (2000). Properties and synthetic applications of enzymes in organic solvents, *Angew. Chem. Int. Ed.*, *39*, 2226-2254.

- [34] 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.
- [35] Micaelo, N. M. & Soares C. M. (2007). Modeling hydration mechanisms of enzymes in nonpolar and polar organic solvents, *FEBS J.*, *274*, 2424–2436.
- [36] 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.
- [37] Serdakowski, A. L. & Dordick, J. S. (2007). Enzyme activation for organic solvents made easy, *Trends Biotechnol.*, *26*, 54–48.
- [38] Rariy, R. & Klibanov, A. M. (1997) Correct protein folding in glycerol, *Proc. Natl. Acad. Sci. U.S.A.*, *94*, 13520–13523.
- [39] Sirotkin, V. A., Hüttl, R. & Wolf, G., (2008). Enzyme-catalysed hydrolysis of L-amino acid esters in a low water organic solvent studied by isothermal calorimetry, *J. Therm. Anal. Calorim.*, *93*, 515–520.
- [40] Sirotkin, V. A. & Faizullin, D. A. (2004). Interaction enthalpies of solid human serum albumin with water-dioxane mixtures: comparison with water and organic solvent vapor sorption, *Thermochim. Acta*, *415*, 127–133.
- [41] Partridge, J., Hutcheon, G. A., Moore, B. D. & Halling P. J. (1996). Exploiting hydration hysteresis for high activity of cross-linked subtilisin crystals in acetonitrile, *J. Amer. Chem. Soc.*, *118*, 12873–12877.
- [42] 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.
- [43] 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.

- [44] McMinn, J. H., Sowa, M. J., Charnick, S. B. & Paulaitis, M. E. (1993). The hydration of proteins in nearly anhydrous organic solvent suspensions, *Biopolymers.*, 33, 1213-1224.
- [45] 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.
- [46] Khmelnitsky, 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.
- [47] Simon, L. M., Kotorman, M., Garab, G. & Laczko, I. (2001). Structure and activity of β -chymotrypsin and trypsin in aqueous organic media, *Biochem. Biophys. Res. Comm.*, 280, 1367-1371.
- [48] Fersht, A. (1999). *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*; Freeman & Co: New York.
- [49] Lehninger, A. L., Nelson, D. L. & Cox, M. M. (1993). *Principles of Biochemistry*; Worth: New York.
- [50] Borisover, M. D., Stolov, A. A., Cherkasov, A. R., Izosimova, S. V. & Solomonov, B. N. (1994). Calorimetric and infrared spectroscopic study of intermolecular interactions of water in organic solvents, *Russ. J. Phys. Chem.*, 68, 48-53.
- [51] Perrin, D. D., Armarego, W. L. F. & Perrin, D. R. (1980). *Purification of Laboratory Chemicals*, Oxford: Pergamon Press.
- [52] Borisover, M. D., Sirotkin, V. A. & Solomonov, B. N. (1995). Thermodynamics of water binding by human serum albumin suspended in acetonitrile. *Thermochim. Acta*, 254, 47-53.
- [53] Sirotkin, V. A. & Kuchierskaya, A. A. (2017). Preferential solvation/hydration of α -chymotrypsin in water-acetonitrile mixtures. *J. Phys. Chem. B.*, 121, 4422-4430.
- [54] 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.

- [55] Sirotkin, V. A. & Kuchierskaya, A. A. (2017). α -Chymotrypsin in water-ethanol mixtures: Effect of preferential interactions. *Chem. Phys. Lett.*, 689, 156-161.
- [56] 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.
- [57] 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.
- [58] Atkins, P. W. (2006). *Physical Chemistry*. 8th ed. Oxford: Oxford University Press.
- [59] Prausnitz, J. M. (1969). *Molecular Thermodynamics of Fluid-Phase Equilibria*. N.J.: Prentice-Hall, Inc., Engelwood Cliffs.
- [60] Belousov, V. P. & Panov, M. Y. (1994). *Thermodynamic properties of aqueous solutions of organic substances*. Boca Raton, Fla.: CRC Press.
- [61] 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.
- [62] Pendin, A.A. (1989). Preferential solvation and thermodynamical properties of nonelectrolyte solutions. *J. Phys. Chem.* 7, 1793-1798.
- [63] Lai, J.T.W., Lau, F.W., Robb, D., Westh, P., Nielsen, G., Trandum, C., Hvidt, A. Koga, Y. (1995). Excess partial molar enthalpies, entropies, Gibbs energies, and volumes in aqueous dimethylsulfoxide. *J. Sol. Chem.* 24, 89-102.
- [64] Tretyakova, T., Shushanyan, M., Partskhaladze, T., Makharadze, M., van Eldik R. & Khoshtariya, D. E. (2013). Simplicity within the complexity: Bilateral impact of DMSO on the functional and unfolding patterns of α -chymotrypsin. *Biophys. Chem.*, 175-176, 17-27.