



ELSEVIER

Biochimica et Biophysica Acta 1545 (2001) 326–338

BIOCHIMICA ET BIOPHYSICA ACTA

**BBA**

www.elsevier.com/locate/bba

## Homotropic cooperative binding of organic solvent vapors by solid trypsin

Valery V. Gorbachuk \*, Marat A. Ziganshin, Nickolay A. Mironov,  
Boris N. Solomonov

*Department of Chemistry, Kazan State University, Kremlevskaya 18, Kazan 420008, Russia*

Received 8 May 2000; received in revised form 23 October 2000; accepted 13 December 2000

### Abstract

Homotropic cooperative binding was observed at vapor sorption of organic solvents (acetonitrile, propionitrile, ethanol, 1-propanol, 2-propanol, nitroethane) by dried solid trypsin from porcine pancreas (0.05 g H<sub>2</sub>O/g protein). The vapor sorption isotherms were obtained by the static method of gas chromatographic headspace analysis at 298 K for ‘vapor solvent+solid trypsin’ systems in the absence of the liquid phase. All isotherms have a sigmoidal shape with significant sorbate uptake only above the threshold of sorbate thermodynamic activity. On the sorption isotherms of non-hydroxylic sorbates the saturation of trypsin by organic solvent was observed above the sorbate threshold activity. The formation of inclusion compounds with phase transition between solvent-free and solvent-saturated trypsin is supposed. Approximation of obtained isotherms by the Hill equation gives the inclusion stoichiometry  $S$ , inclusion free energy, and the Hill constant  $N$  of clathrates. The inclusion stoichiometry  $S$  depends significantly on the size and shape of sorbate molecules and changes from  $S = 31$  mol of sorbate per mol of trypsin for ethanol to  $S = 6$  for nitroethane. The inclusion free energies determined for the standard states of pure liquid sorbate and infinitely dilute solution in toluene are in the range from  $-0.5$  to  $-1.2$  kJ/mol and from  $-3.1$  to  $-8.1$  kJ/mol, respectively, per 1 mol of sorbate. The Hill constants are relatively high: from  $N = 5.6$  for 1-propanol to  $N \approx 10^3$  for nitroethane. The implication of the obtained results for the interpretation of solvent effects on the enzyme activity and stability in low-water medium is discussed. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Enzyme-solvent inclusion compound; Cooperative solid phase transition in enzyme; Molecular recognition by protein; Headspace analysis; Vapor sorption isotherm

### 1. Introduction

The special effect of organic solvents on the selectivity and specificity of enzymatic reactions invoked a lot of questions on the ability of bound solvent to modify the conformation of enzyme active site [1–3]. X-Ray studies of enzyme crystals soaked by organic

solvents do not reveal significant changes in the structure of enzyme active sites compared with the structure of crystals grown from water [4,5]. The changes observed in protein side chain positions due to organic solvent binding by chymotrypsin crystals were supposed to be responsible for the altered substrate specificity of this enzyme [6]. The conformational changes in dry enzymes suspended in non-aqueous medium were also observed by FTIR spectroscopy [7,8]. Another approach to this problem may be the study of cooperative effects at the inter-

\* Corresponding author. Fax: +7-8432-315-416 (work), +7-8432-552-246 (home); E-mail: valery.gorbachuk@ksu.ru