

# CHYMOTRYPSIN-CATALYZED HYDROLYSIS OF HYDROPHOBIC L-AMINO ACID ESTERS IN AN ORGANIC SOLVENT WITH LOW WATER CONTENT AS OBSERVED BY ISOTHERMAL CALORIMETRY

VLADIMIR A. SIROTKIN<sup>1</sup>, REGINA HÜTTL<sup>2</sup>, GERT WOLF<sup>2</sup>

<sup>1</sup>Butlerov Institute of Chemistry, Kazan State University, Kremlevskaya str., 18, Kazan, 420008, Russia  
e-mail: [vsir@mail.ru](mailto:vsir@mail.ru)

<sup>2</sup>Institute of Physical Chemistry, Freiberg University of Mining and Technology,  
D-09596 Freiberg, Germany

The use of enzyme-catalyzed reactions in organic media is highly promising for modern biotechnology. There are many advantages in employing organic solvents for biocatalytic processes, including increased solubility of hydrophobic substrates. However, in general, the enzyme activity in organic solvents is a complex function of the water content in the organic media and the hydration “history” of an enzyme. Hence, an analysis of the thermodynamic and kinetic aspects of biocatalysis in water-organic mixtures appears important in explaining various enzyme activities.

Since practically all biocatalytic reactions have a non-zero enthalpy change, the heat effects accompanying the enzyme-catalyzed reactions in various water-organic mixtures might be a very informative property of the intermolecular processes influencing the activity of enzymes at such unusual conditions. Calorimetry is a reliable method to determine quantitatively this thermodynamic property of a reaction and obtain information about the reaction rate.

In the present work, a new experimental approach based on the combination of calorimetric and spectrophotometric measurements was proposed to study the thermochemistry of enzymatic reactions in organic solvents with low water content. The derivatives of Z-L-tyrosine and Z-L-phenylalanine were used as model hydrophobic substrates. Acetonitrile was used as a model organic solvent.

The molar enthalpy changes of ester hydrolysis and yields of reactions were determined by means of the combined calorimetric and spectrophotometric measurements at 25°C. These results are **the first examples** of the investigations where the molar enthalpy changes accompanying the enzyme-catalyzed reactions in low water organic solvents have been measured by means of isothermal calorimetry. It was found that the molar enthalpy changes of enzyme-catalyzed reactions depend markedly on the buffer ionisation enthalpy changes. On the other hand, the buffer ionisation enthalpy changes depend strongly on the water content in organic solvent. An explanation of the organic solvent effect on the thermochemical characteristics was provided on the basis of the IR spectroscopic data on the state of hydrogen bond network of water in acetonitrile [1]. The results obtained demonstrate that the state of water in organic solvents is an important factor that controls as the reaction heat effects as well as buffer ionisation enthalpies.

## Reference

[1] Sirotkin V.A. et al. J. Structural Chem. 41 (2000) 997-1003.

This work was supported by the “Michail Lomonosov” Programm (a grant of the Ministry of Education and Sciences of the Russian Federation and the German Academic Exchange Service) and the INTAS (Reference No. YSF 2002-34).