Humic substances potentiate inhibitory activity of sulbactam with respect to β-lactamase TEM-1

Kravtsova D., Cherkasova T., Rubtsova M., Grigorenko V., Perminova I.
Lomonosov Moscow State University, Department of Chemistry, Moscow, Russia, d.k.97@mail.ru

Keywords: antibiotic resistance, beta-lactams, synergy, inhibition, humic substances

doi: 10.36291/HIT.2019.kravtsova.093

Antibiotic resistance became a global problem, while microorganisms developed several adaptive tools to escape the action of antibiotics. The most efficient tool, which is used by gram-negative bacteria, is enzymatic degradation of antibiotics. It is realized via release of beta-lactamases which are capable of degrading beta-lactam ring of the most powerful beta-lactam antibiotics. To overcome this problem, inhibitors of beta-lactamases are widely used in clinical practice. However, they are quickly loosing activity due to efficient molecular recognition developed by microorganisms. The aim of this work was to test humic substances (HS) as a new type of beta-lactamase inhibitors. The ideas behind were: firstly, HS are capable of interaction with a broad range of enzymes and this might reduce activity of beta-lactamases; secondly, molecular ensemble of HS has dynamic constitution which might hinder molecular recognition of HS or embedded antibiotics by bacteria. For achieving the goal of this study, we have synthesized CENTA, which is a chromogenic analogue of beta-lactam antibiotic cephalothine as described in [1]. We used CENTA as a chromogenic substrate for studying enzymatic activity of β-lactamase TEM-1 in the presence of sulbactam (clinically approved beta-lactam inhibitor) and humic materials used in this study. A set of humic materials used for testing was assembled from the samples differing substantially in hydrophobicity and functional group composition. It included coal hymatomelanic acid (CHM-Irk), coal humic acid (CHA-Irk), coal humic acid (CHP), hydroquinone derivative of CHP (CHP-HQ), and peat fulvic acid (PFA-HT).

The parameters of enzymatic kinetics of CENTA hydrolysis by the TEM-1 β-lactamase were determined. The values of KM_{eff} and V_{max} accounted for (89 ± 1) μM and (1.9 ± 0.1) μM/s, respectively. The inhibition constant (KI) of the enzymatic hydrolysis of CENTA by sulbactam accounted for (12.7 ± 2.5) μM. A competitive type of inhibition was established. According to the data obtained, the most active was a mixture of sulbactam and CHA-Irk, which reduced initial hydrolysis rate by 42%. The presence of CHA-Irk enhanced activity of sulbactam two times.

The second place in potentiating inhibition efficiency of sulbactam was taken by CHP-HQ, whose presence inhibited the reaction by 39%. It was followed by CHA-Pow and CHM-Irk, which reduced the hydrolysis rate of CENTA by 31% and 30%, respectively. Fulvic acids (PFA-HT) had the lowest synergetic activity – the resulting inhibitory activity accounted for 23%, which is very close to the activity of pure sulbactam (21%).

The results obtained are indicative of synergetic action between humic substances and low molecular weight β-lactamase inhibitors. The value of the found synergetic effect was the highest for the most hydrophobic coal humic materials, and the lowest one – for the hydrophilic fulvic acids.

Acknowledgement. This research was partially supported by the Russian Foundation for Basic Research (grant 18-29-25065).

References

1. Bebrone C. et al. // Antimicrob Agents Chemother. 2001. 45:1868–1871.