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Peculiarities of the Mechanism of Interactions of Catalytic Antibodies with Organophosphorus Substrates

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Abstract—Catalytic antibodies are a promising model for creating highly specific biocatalysts with predetermined activity. However, in order to realize the directed change or improve their properties, it is necessary to understand the basics of catalysis and the specificity of interactions with substrates. In the present work, a structural and functional study of the Fab fragment of antibody A5 and a comparative analysis of its properties with antibody A17 have been carried out. These antibodies were previously selected for their ability to interact with organophosphorus compounds via covalent catalysis. It has been established that antibody A5 has exceptional specificity for phosphonate X with bimolecular reaction rate constants of 510 ± 20 and 390 ± 20 $min^{-1}M^{-1}$ for kappa and lambda variants, respectively. 3D-Modeling of antibody A5 structure made it possible to establish that the reaction residue L-Y33 is located on the surface of the active site, in contrast to the A17 antibody, in which the reaction residue L-Y37 is located at the bottom of a deep hydrophobic pocket. To investigate a detailed mechanism of the reaction, A5 antibody mutants with replacements L-R51W and H-F100W were created, which made it possible to perform stopped-flow kinetics. Tryptophan mutants were obtained as Fab fragments in the expression system of the methylotrophic yeast species *Pichia pastoris*. It has been established that the effectiveness of their interaction with phosphonate X is comparable to the wild-type antibody. Using the data of the stopped-flow kinetics method, significant conformational changes were established in the phosphonate modification process. The reaction was found to proceed using the induced-fit mechanism; the kinetic parameters of the elementary stages of the process have been calculated. The results present the prospects for the further improvement of antibody-based biocatalysts.

Keywords: catalytic antibodies, organophosphorus compounds, induced-fit mechanism
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INTRODUCTION

Today, antibodies are widely used in medicine and biotechnology due to their ability to specifically recognize and bind antigens. The possibility to artificially create antibodies against various compounds that have no natural analogs is one reason why this area of science receives constant attention. However, the functions of antibodies are not limited to binding antigens. In the late 20th century, the capacity of antibodies for biocatalysis, which allows them to both bind and destroy antigen, was discovered [1]. Currently, a considerable amount of catalytic antibodies (abzymes) that catalyze various chemical reactions, for which no natural enzymes are known, has been produced [2]. In connection with this, the application of catalytic antibodies for neutralizing synthetic toxins creates broad prospects in the treatment of chemical poisoning, in particular organophosphate (OP) poisoning.

Earlier, the screening of a semi-synthetic library of human immunoglobulin variable fragments using *p*-nitrophenyl-8-methyl-8-azabicyclo[3.2.1]octa phenylphosphonate (phosphonate X) enabled the selection of a panel of recombinant single-chain antibodies capable of covalent interactions with organophosphates. Antibodies A5 and A17 exhibited the highest activity; L-Tyr33 and L-Tyr37 residues of A5 and A17, respectively, were found to be covalently modified [3]. The difference in the position of the key amino-acid residue in the reaction center evidences the possible implementation of different mechanisms of interactions between the abzymes and the substrate. Anti-

Abbreviations: CDR, complementarity determining region; FRW, framework region of the antibody variable domain; V_H, variable domain of heavy chain; V_L, variable domain of light chain; QM/MM, hybrid quantum mechanics/molecular mechanics modeling; OP, organophosphates.