ABZYMES - A CHALLENGE FOR THE MEDICAL BIOTECHNOLOGY AND PUBLIC HEALTH

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Keywords: abzyme, antibody, antigen, abzyme vaccine, engineered antibody, enzyme, epitope, substrate transition state analog, biopharmaceuticals, biosimilars

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Summary

A new branch of the Medical biotechnology – abzyme biotechnology is emerging. Abzymes are antibodies with catalytic activity. They combine the specificity and substrate turnover efficiency of the enzymes with the selective targeting of the antibodies. The catalytic antibodies have been developed for catalysis of almost all reactions occurring in the living systems and even for catalysis of kinetically and thermodynamically unfavorable reactions.

The catalytic antibodies are part of the antibody's repertoire *in vivo*. Natural antibodies have been found in healthy people, in patients with clinical autoimmune syndrome as well as in individuals resistant to AIDS. The strategies for obtaining abzymes are discussed.

The unique characteristics of abzymes provide their great practical relevance. The abzymes which recognize, bind and transform target cell or molecule selectively would be therapeutically valuable.

Synthesis of specific catalytic Abs, able to neutralize drugs and toxins would destroy them into circulation, before initiating toxic effects on nervous system and other tissues.

The first potential anti-drug vaccine was developed by immunization with a stable transition state analog of cocaine. In 2008, Abzymes neutralizing HIV by destroying its T-cell attachment site have been designed. The potency of this abzyme revives the hopes for its clinical application as immunotherapeutic and topical microbicide reagent.

The identification of IgMs and recombinant Ig fragments that hydrolyze amyloid β-peptide provides a foundation for the development of catalytic antibodies for treatment of Alzheimer's disease.

Engineered bispecific Abs are suitable for antibody directed enzyme prodrug therapy – activation of the prodrug at the moment of its delivery to the target cells, tissues or organs. Catalytic antibodies are a great challenge for the medical biotechnology.

Engineered Abzymes with prescribed catalytic and cytotoxic activity belong to a new generation of diagnostic and therapeutic tools that will enrich the list of biopharmaceuticals.

1. Introduction

The biological drugs – medicines isolated from living organisms have been used in therapy for a long time. The advances made in medical biotechnology enrich the list of diagnostic tools and medicines for prophylactics, prevention and therapy. Many enzymes, proteins, hormones, antibodies, vaccines are produced by biotechnological methods.

The discovery of the catalytic antibodies called *abzymes* [or *catmab* – catalytic monoclonal antibody] in 1886, which combine the properties of the enzymes and antibodies, drives the research for the design of a new generation of effective and highly specific therapeutical agents in prevention and treatment of cancer, drugs dependence and drug abuse, virus infections, for manipulating AIDS pandemic.

In June 2008 the scientific group of Sudhir Paul UT Huston laboratory announced that a new strategy for attacking HIV has been developed – to destroy by abzymes the Achilles heel of HIV – the cellular attachment site of the envelope protein gp120, which is constant in all known HIV strains. In that way the virus could not infect target cells T helper cells of the immune system.

2. Structure and Function of Enzymes

Biochemical transformations in the living cells are catalyzed by biological catalysts. Most biological catalysts are proteins. Nucleic acids also can catalyze some reactions. Catalytic proteins are referred to as enzymes and catalytic ribonucleic acids as ribosymes.

Catalysts are substances that increase the rate of chemical reactions. The reactions proceed when the products have less chemical energy than the reactants. Reactions tend to proceed until equilibrium is reached. The catalyst enzymes do not affect the position of equilibrium of a reversible reaction. Many substances, although capable of reacting,

do not actually do so. The reason for that is that reactants do not have enough energy to react at normal temperature. There is an energy barrier – activation energy.

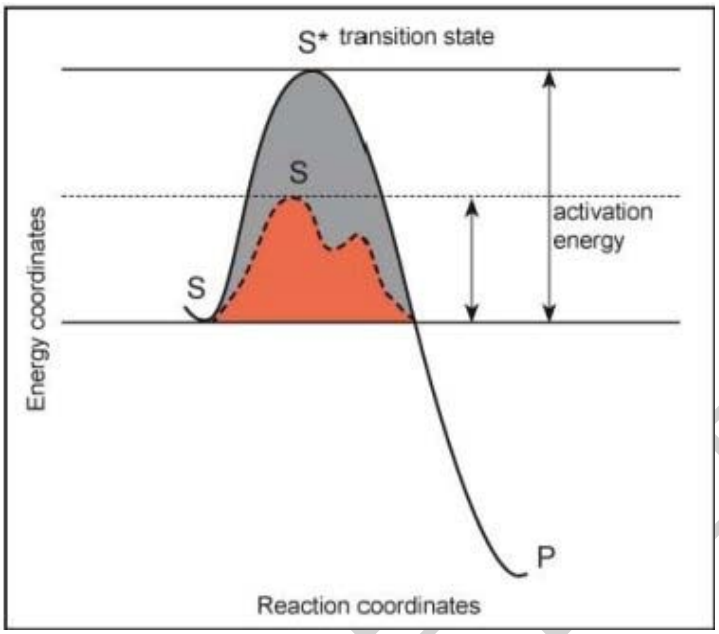

Figure 1. Enzymes accelerate the chemical reactions by reducing the activation energy

The reacting molecules pass trough a transition state – the state with higher energy than the energy of the reactants. The enzymes speed up the reaction, like other catalysts, providing alternative pathway for the reaction through another transition state with lower activation energy.

The enzymes are proteins – polypeptides made by linking together hundred or even several thousand amino acid monomer units by peptide bonds. The polypeptide chain folds in a unique three-dimensional structure. The folding depends on the number and the sequence of the amino acids of the polypeptide chain that is genetically determined. The specific folding in a three dimensional structure referred to as tertiary structure creates surfaces with precise shapes. The so formed pocket, cleft or crevice on their enzyme surface is called the active site (center) where catalysis takes place. Several amino acids in the active site take part in the positioning; binding of the substrate and in the catalytic act. The mode of action of an enzyme can be represented as follows:
$E + S \leftrightarrow ES \rightarrow E + P$

The substrate (S) binds into the enzyme (E) active site forming an enzyme substrate complex (ES). The substrate is transformed into the product (P) that dissociates from the enzyme. The enzymes are very efficient catalysts. A single enzyme molecule can transform many substrate molecules. The chemical reaction that involves breaking and forming of chemical bonds takes place in the active site.

Over 100 years ago, Emil Fisher suggested that the substrate binds to the active center in a manner similar as a key fits into a lock. Only substrates that are complementary in

shape and chemical groups (and charge) to the active site can fit to it. Actually, the enzymes are highly specific – an enzyme can bind and transform one or several similar substrates. The "lock and key" concept was changed and developed. It becomes clear that both enzyme and substrate are flexible. When an ES is formed the substrate induces the enzyme protein molecule to fit around it. The "induced fit" model is now generally accepted.

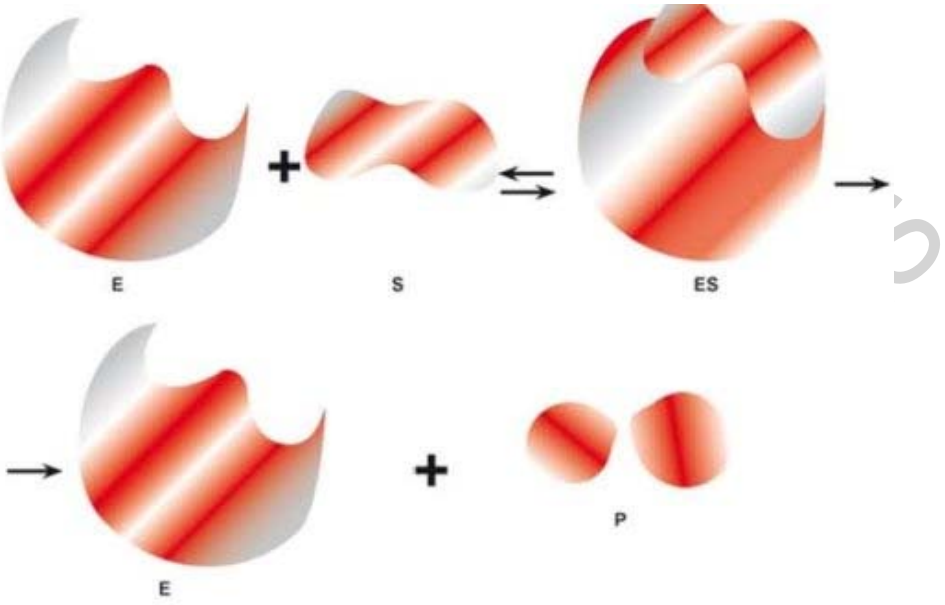

Figure 2. Schematic representation of an enzyme reaction

In 1940s Linus Pauling suggested that the active center of an enzyme is complementary not to the substrate itself but to its transition state (see figure 2). The enzyme stabilizes the transition state of the substrate and pushes the reaction to proceed. He proposed also that an antibody raised to the transition state of a substrate should have catalytic activity.

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Bibliography

Gabibov A.G. (2006). Antibody catalysis: Biochemistry, immunology and pathology, *Immunology letters*, 103, 1-2. [This points the periods in development of the enzymology and perspectives for biomedical application of abzymes].

Gabibov A.G., Ponomarenko N.A., Tretyak E.B., Paltsev M.A., Suchkov S.V. (2006). Catalytic

antibodies in clinical autoimmunity and modern medicine, *Autoimmunity reviews*, 5, 324-330. [This is a comparison of the data of different scientific groups on the nature of abzymes, their relevance for autoimmune disorders and their potential value for medical science and public health].

Hudson J., Souiriau C. (2003). Engineered antibodies, *Nature Medicine*, 9, 129- 134. [This reviews the experimental approaches for engineering intact antibody molecules and Ab fragments and their utility for application in the diagnostics and treatment].

Lacroix-Desmazes S., Wootla B., Delignat S., Dasgupta S., Nagaraja V., Kazatchkine M., Kaveri S.V. (2006). Pathophysiology of catalytic antibodies, *Immunology letters*, 103, 3-7. [This represents the approaches for raising abzymes and reviews the data for occurrence of natural catalytic antibodies and their possible biological significance].

Nevinski G.A. Favorova O.O., Buneva V.N. (2002). Catalytic antibodies: new characters in the protein repertoire, *Protein-protein Interaction: A molecular cloning manual*, 523-534, Cold Spring Harbor Laboratory press. [This discusses the methods for isolation of natural catalytic antibodies and production of "artificial" abzymes, the quality control criteria for intrinsic catalytic activity and structure-function relationship].

Paul S. Nichiyama, Planque S., Taguchi H. (2006). Theory of proteolytic antibody occurrence, *Immunology letters*, 103, 8-16. [The paper provides the theoretical aspects of the abzymology that will stimulate medical and basic science advances in the field].

Planque S., Nishiyama Y., Taguchi H, Salas M., Hanson C, Paul S. (2008). Catalytic antibodies to HIV: physiological role and potential clinical utility, *Autoimmunity reviews*, 7, 473-479. [This presents the data of Paul's laboratory on catalytic antibodies to the T-cell attachment site of HIV and discusses the suitability of the conserved HIV epitope as a target for immunotherapy and prophylactic vaccination].

Xueyong Zhu, Dickerson T.J., Rogers C.J., Kaufmann G.F., Mee J.M., McKenzie K.M., Janda K.D., Wilson I.A., Complete reaction cycle of a cocaine catalytic antibody at atomic resolution, *Structure*, 14, 205-216. [This announced the complete reaction cycle of a cocaine catalytic antibody that is designed for treatment of cocaine craving addiction and overdose by using the abzyme].

Taguchi H., Planque S., Nishiyama Y., Szabo P., Weksler M.E., Friedland R.P. Paul S. (2008). Catalytic antibodies to amyloid β-peptide in defense against Alzheimer disease, *Autoimmunity reviews*, 7, 391-397. [It announced the identification of IgMs and recombinant Ig fragments hydrolyzing amyloid β-peptide that is under clinical trials for immunotherapy of Alzheimer disease].

Tredree R. (2008). Progress with biopharmaceuticals in Europe. *Hospital pharmacy Europe*, 41, 21-24. [This provides an overview on current status and developments on biopharmaceuticals and biosimilars in EU for last decade].

Biographical Sketches

Ilko Getov was born in Sofia, Bulgaria and graduate master of pharmacy at the Faculty of Pharmacy in 1988. He obtained PhD in Pharmacoepidemiology and pharmacovigilance at Medical University Sofia. He has appointed to the staff of the department of Social pharmacy in 1996 and received Associate professorship in 2005. He specialized in post marketing surveillance and drug regulation in Germany, Italy, etc. Ilko Getov gives lectures on Social pharmacy, Pharmacoepidemiology and Clinical pharmacy. He published original scientific papers in international and local journals and has several textbooks for pre- and postgraduate students. He is a member of Global Development Committee at International Society of Pharmacoepidemiology, European Society of Clinical Pharmacy and EURODURG, joint the WHO Drug policy monitoring project – level II in 2003-2004, acting as evaluator of the projects for EU LLL Program. At national level he is a Chair of Expert commission on medicines advertising at Bulgarian drug agency, member of a Supreme Pharmacy Council at the Minister of health, etc. Mr. Getov participates as a lecturer in Pharmacoepidemiology in post-graduated courses at the Faculty of Pharmacy, University of Belgrade, Serbia. He is married and has one daughter.

Trenka Argirova was born in the city of Panagurishte, Bulgaria. Graduated in biology at the University of Sofia she received a PhD degree in biochemistry. She was appointed to the staff of the Department of Biochemistry of Sofia University in 1966 and specialized at Courtauld Institute of Biochemistry in London and in Moscow University. Her early investigations and original papers published are in the field

of protein synthesizing apparatus of the cells and later on in the biochemistry of autoantibodies in autoimmunity. As an Associated Professor in biochemistry she has been giving lectures in Biochemistry and in Metabolic regulations for students in Bachelor and Master Degree programs at University of Sofia. She has published text books and manuals in biochemistry and immunochemistry. Member is of the Bulgarian Society of Biochemistry, Biophysics and Molecular Biology and joint member of the Federation of the European Biochemical Societies. Argirova participated in the organization of the training courses on biochemical education and TEMPUS projects.