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Catalytic antibodies as potential therapeutics

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The idea that enzymes can be generated by employing antibodies complimentary to haptenic groups, resembling the transition state of a given reaction, laid down the foundation of catalytic antibodies. New possibilities arise for their therapeutic applications, because of high degree of reaction specificity, greater affinity towards transition state analog and the latent ability to block unwanted protein-protein interactions. Antibody directed abzyme prodrug therapy (ADAPT) will largely replace antibody directed enzyme prodrug therapy (ADEPT) for selectively delivering chemotherapeutic agents to the affected tissues. They can enzymatically cleave specific surface proteins and sugars on viruses or tumour cells, thereby disrupting the invaders. They had also been successfully used to detoxify drugs like cocaine and methamphetamine. Desired reaction selectivity can be induced in antibodies to exhibit a wide range of chemical reactions, so that they would prove to be a milestone in human endeavour to treat genetic diseases, which show brighter prospects for their therapeutic applications in near future. This review aims at analyzing the updated information on biochemical and mechanistic implications of catalytic antibodies with special reference to their therapeutic application and the advances made in these areas.

Keywords: Abzyme; antibodies; hapten; transition state; therapeutics

Introduction

In 1966, I L Slobin reported the preparation of antibodies which carried out specific hydrolysis of ortho nitrophenyl esters giving an indication that antibodies could have catalytic properties[1]. But in 1969 several research works proposed the synthesis of an enzyme by employing antibodies complementary to a haptenic-group (A small molecule, which can elicit immune response when attached to a large carrier such as protein)[2] that resembles the transition state of a given reaction (Unstable highest energy reaction intermediate)[3]. This laid the foundation of catalytic antibodies, later also known as abzymes[4]. Since then researchers around the world have endeavoured to exploit the diversity of the immune system to engineer antibodies with novel catalytic functions.

Many workers succeeded in producing monoclonal antibodies with catalytic properties, which can catalyze the hydrolysis of esters and amides; 38C2 was the first commercially available catalytic antibody. These antibodies with catalytic properties showed high level of substrate specificity and are inhibited by the antigens, which were used as transition state analogue in their production[5-7]. The proof that catalytic antibodies are responsible for any observed catalytic reaction was given by the inhibition of catalytic antibodies by the transition-state analog hapten[8]. This was based on the observations made by Linus Pauling[9] in 1946 that enzymes achieve catalysis through being tuned to complement and thereby stabilize the high-energy transition state of a reaction. Antibodies can also acquire catalytic properties by natural means. These have been isolated and evaluated like certain DNA and RNA hydrolyzing antibodies, which were isolated from sera of patients with systemic lupus erythematosus[10]. A systemic autoimmune disorder and vasoactive intestinal peptide hydrolysing antibodies were also isolated from the serum of bronchial asthma patients[11].

Catalytic antibodies offer new possibilities for their potential therapeutic applications because of a high degree of reaction specificity, greater affinity towards transition state analog and their latent ability to block unwanted protein-protein interactions. This review aims at analyzing the updated information on mechanical, biochemical and therapeutic aspects to figure out the potential of this novel biocatalyst as a powerful therapeutic.

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Mechanism of Antibody Catalysis

Recent NMR and crystallographic studies on immunoglobulins are notable for providing valuable insights into the structural basis of antibody catalysis. According to which catalytic antibodies are known to bind very tightly to the transition state analog haptens that were used to produce them during the immunization process; the binding forces within the antibody binding sites are enlisted to stabilize transition states and intermediates, thereby lowering a reaction's energy barrier and increasing its rate. Transition-state analog haptens can interfere with the catalytic reaction by binding in the antibody-binding pocket, thereby preventing any substrate molecules from binding and reacting; this can occur only when the antibodies have a binding site that is complementary to a transition state or intermediate structure in terms of both 3-D geometry and charge distribution. This encourages the substrate to adopt a transition state like geometry and charge distribution, due to which unwanted products can be prevented and, thus, reaction selectivity can be increased[12-13]. Whereas enzymes accelerate reactions by providing a reaction pathway, in which the transition state has a lower free energy. Hence, product is more rapidly formed than in the uncatalyzed reaction. They obtain much of their catalytic efficiency from tight binding of the transition state for the reaction. This binding energy stabilizes the transition state of substrate, which reduces the activation energy for the chemical modification of bound substrate. Thus, by decreasing the free energy of activation of chemical reactions, enzymes serve as catalyst[14].

Rate of reaction catalyzed by a catalytic antibody is measured by kinetic parameters[15], such as, K_M and V_{max}. Catalytic antibodies have a low K_M, indicating that they readily bind a target molecule, but have low V_{max} values, which indicate a slow reaction rate. However, the rate of reaction catalyzed by them are up to a million fold greater than the corresponding uncatalysed reaction but in many cases, catalytic antibodies have not yet approached the rates of reaction catalyzed by natural enzymes. Since real enzymes typically change their own conformation in binding the transition state, this do not necessarily happen with catalytic antibodies as they adopt an "induced fit" binding mode that lead to enhanced complementarity between the antibody combining site and the hapten (Table 1)[16-29].

Catalytic Antibodies *Vs* Enzymes

One of the most important advantages of catalytic antibodies over enzymes is that the desired reaction can be selectivity programmed into the antibodies by using an appropriately designed hapten, which is not possible with enzymes. Catalytic antibodies almost always demonstrate a high degree of substrate selectivity. In addition, such catalytic antibodies produced have sufficient regioselectivity to give a single product for a reaction.

They can be produced by immunization with a single-handed version (only left-or only right-handed) of a hapten, and only substrates with the same handedness can act as substrates for the resulting catalytic antibodies. The net result is that a high degree of stereoselectivity is observed in the antibody-catalyzed reaction[30].

Therapeutic Potential

From ADEPT to ADAPT

Taking cue from the binding specificity of antibodies, antibody directed enzyme prodrug therapy (ADEPT) was developed that had exploited the binding specificity of the antibodies for targeting the chemotherapeutic agent to affected tissue, which was particularly advantageous in case of cancer chemotherapy to minimize non-specific toxicity associated with chemotherapeutic agents. This therapy is realized in two steps. First, the administered enzyme conjugated antibody combines with the cancer cell, followed by administration of prodrug that is activated in the vicinity of the cancer cell by the enzyme to release the parent drug, which would then show its cytotoxic effect. Apart from drug immunoconjugates in which a prodrug is coupled to antibody, various other approaches have also been followed like radioimmunoconjugate, in which the

Table 1—Some of catalytic antibodies whose crystallographic structures are known

Antibody	Reaction catalyzed	PDB code
McPC603	Ester hydrolysis	1MCP
39A11	Diels Alder	1A4K
13G5	Exo-Diels Alder	1A3L
7C8	Chloroamphenicol hydrolysis	1CT8
Jel103	Nuclease	1MRE
Jel42	Peptidase	2JEL
43C9	Amide Hydrolysis	1MIG
1F7	Chorismate mutase	1FIG
5C8	Cyclase	25C8
17E8	Esterolytic	1EAP
7G12	Chelation	3FCT
28B4	Oxygenation	1KEL

radioisotope is coupled to an antibody. In an immunotoxinconjugate, a toxin molecule is coupled to an antibody. But one of the major limitations of such immunoconjugates is the immunogenicity associated with the conjugate component. Since they are mostly bacterial toxins and bacterial enzymes are used to activate the prodrug, they are comparatively more specific than human enzymes[31-32]. Thus, by using humanized catalytic antibodies, unwanted immunological reactions can be effectively minimized.

Through antibody directed abzyme prodrug therapy (ADAPT), the latent ability of catalytic antibodies to block unwanted protein-protein interactions due to their high degree of reaction specificity is being exploited to activate the prodrugs. A number of examples exist in which pharmaceutically active compounds are administered as prodrugs. Here a catalytic antibody can be used to convert a prodrug into an active compound. 6D9 was the first antibody, reported in 1993, which was used for activation of ester prodrug to chloroamphenicol with a rate enhancement of 1.8×10^3. After that many antibodies were tested for prodrug activation, like the catalytic antibody 38C2, which was successfully tested to deliver insulin in a prodrug form by catalyzing the cleavage of insulin molecules modified with aldol-termination into organoinsulin *in vivo*. In this process, the catalytic aldolase antibody 38C2 specifically recognizes and cleaves aldol-terminated linkers in the prodrug, restoring native insulin in order to control its biological activity *in vivo*[33].

Catalytic antibodies have been effectively used for specific targeting of cancer cells to destroy the malignant tissue without damaging normal surrounding tissues. Cancer cells contain unique determinants called tumour cell antigens on their surface that are lacking in normal cells. Thus, by utilizing antibodies that specifically bind to these tumour cell antigens, anticancer drugs can be delivered directly to the tumour cells. Scientists have developed antibodies with two distinct antigen-binding sites. One site binds with high affinity to a tumour cell antigen, while the second site catalyzes the cleavage of a prodrug (catalytic antibody 38C2 HPMA-copolymer conjugate). First, the antibody is administered to patients once it binds to the tumour cells with high affinity. Then, the prodrug is introduced into the bloodstream, which gets activated only in the vicinity of the targeted antibodies. Healthy cells are spared from the toxic effects of cancer drugs by this technique, which again highlights one of the most important features of catalysis by antibodies that the desired reaction selectivity can be programmed into the antibody by using an appropriately designed hapten[34].

Vaccination Against Weight Gain and Drug Addiction

Conventionally, vaccines were used to get protection against infectious diseases, but now they have been reported to be used against weight gain and drug addiction. Recently, some workers have reported vaccines to treat obesity; they have generated antibodies which can catalyze degradation of Ghrelin; an endogenous ligand for the growth hormone secretagogue receptor, playing a vital physiological role in energy homeostasis and weight gain[35]. Monoclonal antibodies like murine mAb 15A10 and YX1-40H10 are generated to detoxify drug of abuse, cocaine and methamphetamine, respectively. Murine mAb 15A10 degrade cocaine into the resulting nontoxic products, ecgonine methyl ester and benzoic acid. Thus, by cleaving specific bonds they can eliminate toxic effects of drugs[36-37].

Anti-nicotine catalytic antibodies have also been developed. These are generated by using analog of the nicotine ground state that degrades nicotine with the help of singlet oxygen generated by riboflavin and visible light[38]. Catalytic antibodies can be envisaged to become a universal tool for detoxifying various toxic agents ranging from organophosphorus insecticides to biological weapons like nerve gas.

Antimicrobial Catalytic Antibodies

Catalytic antibodies can also be employed for carrying out sequence specific cleavage of peptides or carbohydrate associated with the viral or bacterial coat[39]. Anti HIV proteolytic antibodies were raised to carry out catalytic cleavage of gp120, by immunization with an electrophilic analog of gp120 that expresses enhanced nucleophilic reactivity and accounts for their ability to form irreversible adducts with gp120 *via* nucleophile-electrophile mechanisms and supporting water attack on the covalent adduct, resulting in catalytic cleavage of gp120, which distorts 3D structure of the envelope protein sufficiently to interfere with the binding of the virus to CD_4+ cells[40].

Catalytic Antibodies to Treat Genetic Diseases

Catalytic antibodies would prove to be a milestone in human endeavour to treat genetic diseases. By immunizing with an appropriate antigen the production of an abzyme could be elicited, whose function would replace that of the missing enzyme. Thus they can be used to treat deficiency of an extracellular enzyme[41].

Other Therapeutic Applications

Catalytic antibodies have been reported to be very much effective in slowing down the development of neurodegeneration. This is associated with multiple sclerosis by site-specific degradation of a neural antigen acting as epitope for auto-antibodies[42]. In a number of clinical studies, catalytic antibodies have been found to be effective in bringing down sepsis related death through the removal of metabolic wastes and thereby protecting against infection[43].

Non-therapeutic Applications

Considerable progress has been realized towards eventual utility of the catalytic antibodies. Many different examples of such catalytic antibodies have been reported like proton transfer redox reaction, enantioselective reactions, beta elimination, ester hydrolysis, amide hydrolysis, amide bond formation, transestrification, photo-induced cleavage, photo-induced dimerization, decarboxylation, claisen rearrangement of chorismic acid to prephenic acid, bimolecular amide bond formation, 6-membered ring lactonization and even Diels-Alder reaction[44-46]. Antibody 39-A11, which was generated against the bicyclo [2.2.2] octane hapten, has been shown to catalyze the Diels-Alder reaction of a diene with dienophile to give cyclohexene. This shows that catalytic antibodies are not limited to chemical transformations with enzymatic precedent[47]. Selenium-containing anti-oxidant catalytic antibodies have been also developed to protect cultured epidermal cells from ultraviolet B (UVB) light injury. These antibodies catalyzed the reduction of H_2O_2, a reactive oxygen species produced by UV irradiation[48].

Like enzymes, catalytic antibodies have also been reported to be immobilized and the results indicate enhanced stability in organic solvent. Lipase like catalytic antibodies retain the same activity and stereospecificity, which they exhibit in free solution; when bound to inorganic support[49]. Potentiometric biosensors have been successfully developed by using catalytic antibodies, wherein a micro-*p*H electrode is modified with a catalytic antibody 20G9, which catalyzes the hydrolysis of phenyl acetate, producing hydrogen ions that can be monitored by the electrode[50].

Approaches Towards Production of Catalytic Antibodies

Production of catalytic antibodies calls on knowledge at the crossroads of various fields. Ranging from general protein chemistry to enzymology and immunology, as well as protein-protein interactions, for which the scope of applications is wide. There are various approaches towards production of catalytic antibodies, such as, transition state analogue approach, bait and switch approach, reactive immunization approach and anti-idiotypic antibody approach.

Generating monoclonal antibodies to a transition state analogue by immunizing mice and screening for antibodies with catalytic activity has made first generation catalytic antibodies. One of the major obstacles in acquiring catalytic antibodies is that it requires labour-intensive procedures to select catalytic antibodies from huge repertories of antibodies. These have been shown to catalyze several chemical reactions. This is one of the reasons why catalytic antibodies have relatively low turnover rates and cannot compete with the naturally occurring enzyme counterparts. As a consequence, catalytic antibodies have not previously achieved prominence as therapeutic or diagnostic tools.

Immunizing a mouse with a transition state analog is by definition inefficient. This is because it selects B cells on the ability of surface immunoglobulin to bind the analogs and not on the catalytic activity of the surface immunoglobulin. This approach has a severe limitation. It is difficult to predict the structure of transition state analogs; also true transition states for most reaction intermediates are unstable. Thus, true transition states or intermediates cannot be isolated or used as haptens for immunization; instead so-called transition-state analog molecules are used to the extent that the transition-state analog molecule resembles a true reaction transition state or intermediate. The elicited antibodies will also be complementary to that transition state or intermediate and, thus, lead to the catalytic acceleration of that reaction. But some times antibodies capable of catalytic peptide bond hydrolysis are also likely to inactivate target antigens. This occurs due to inefficient screening method of antibodies. As a result, along with favourable antibodies, some unwanted antibodies remain attached inadvertently. Such catalytic antibodies would be invaluable as therapeutic agents to selectively hydrolyse protein or glycoprotein coats of viruses, cancer cells or other physiological targets. But with the development of methods like catELISA[51], which allows screening of catalytic antibodies directly in hybridoma growth medium, allows screening of large number of clones of potential catalytic antibodies more efficiently than conventional methods which utilizes binding affinity of antibodies towards transition state analogue.

Antibodies are raised against the enzyme's active site, which are known as idiotypic antibodies; a second set of antibodies are produced against the antigen-binding site of these idiotypic antibodies that act as replica of enzyme active site and are known as anti-idiotypic antibodies. Anti-idiotypic approach for producing catalytic antibodies can, thus, be used as an alternative to the anti-transition state analogue approach. A monoclonal antibody IgM 9A8, which was obtained as an antiidiotype to AE-2 mAb, is a known inhibitor of acetylcholinesterase and displayed esterolytic activity[52]. Some studies have also suggested antibodies present in patients with autoimmune disorder are produced by same antiidiotypic approach[53].

Catalytic activity can also be induced in antibodies by attaching reactive groups to key locations of binding sites, which are optimal for binding the transition state, and catalyzing the reaction with the substrate by carefully selecting transition state analog with X-ray crystallographic techniques. Thus, only imagination can limit the diversity of reactions shown by this novel catalyst[54-55].

Conclusion

Optimum biodistribution along with high degree of reaction selectivity leads to blocking of unwanted protein-protein interaction, which will establish catalytic antibodies as future panacea. The development of economically viable and robust techniques for the screening of catalytic antibodies, together with site directed mutagenesis and homology modeling will provide further details about their structure and catalytic mechanism of a given antibody. With more technological advancements and research, catalytic antibodies will surely emerge as potential therapeutics.

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