# Teaching Antibodies New Tricks

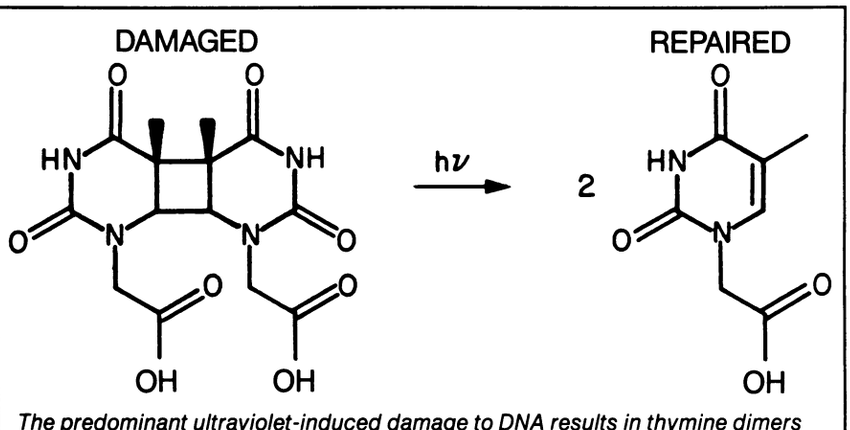
## *Antibodies that act like enzymes are filling chemists' heads with new visions*

By IVAN AMATO

In a scene from the 1966 science-fiction film "Fantastic Voyage," Raquel Welch finds herself the target of Hollywood-style antibody molecules that attack her like homicidal pythons. Never mind that real antibodies would be invisible even to a cell-sized Welch. Never mind that real antibody responses to first-time invaders take days to develop and that antibodies normally bind to their targets in entirely passive encounters. A bit shaken, Welch survives the attack.

Lucky for her the film wasn't made 20 years later. That's when two research groups not far from Hollywood showed the world how to make antibodies that not only bind to specific targets but also behave like catalysts, chemically rearranging them. With this in mind, the screenwriter for a new version of the movie might have the monstrous antibodies stick Welch's hands together or even lop off her leg in a molecular version of "Jaws."

These enzyme-like "catalytic antibodies" may soon make the transition from laboratory curiosities to sophisticated molecular tools for a vast repertoire of important medical, environmental and industrial jobs, including cutting through viral protein coats, deactivating toxic chemicals and creating drugs with fewer side effects. In preliminary experiments, researchers already have made catalytic antibodies that repair DNA damage caused by exposure to solar ultraviolet radiation, break the peptide bond between two specific amino acids or transform one end of a linear molecule into a ring structure. These accomplishments represent a mere peek into a warehouse of potential applications, researchers say.




Schultz/Univ. of Calif., Berkeley

*The predominant ultraviolet-induced damage to DNA results in thymine dimers (left), made of two thymine units joined like Siamese twins. Catalytic antibodies and a dose of light energy (hν) repair the damage by splitting the dimer into individual thymine units (right). Thymine is a building block of DNA.*

Enzymes are proteins that hasten, or catalyze, difficult and intricate biochemical transformations. Unassisted, such reactions would occur far too slowly to sustain life or would arise only under specialized laboratory conditions. Enzymes work by binding molecules and lowering the amount of energy needed to trigger specific chemical changes. For each of the thousands of vital reactions in the world of the living, there is an enzyme nearly perfectly suited to catalyze it.

Antibodies are immune system proteins that, like enzymes, bind to target molecules with intimate specificity. But unlike enzymes, they don't normally catalyze reactions. Their biological responsibility involves simply sticking to and flagging the often-threatening bacterial, viral or molecular outsiders called antigens. Other elements of the immune system, such as white blood cells, then know where to attack.

In December 1986, two independent research groups ushered antibodies into a new arena of more active applications, including out-of-body chemical tasks. Teams led by Richard A. Lerner of the Research Institute of Scripps Clinic in La Jolla, Calif., and Peter G. Schultz of the University of California, Berkeley, simultaneously reported they had commandeered the mammalian immune system to manufacture antibodies that act like tailor-made enzymes.

"The nice thing about this is that you make nature give you the selectivity you want," notes chemist and molecular receptor specialist Donald J. Cram of the University of California, Los Angeles.

The first catalytic antibodies reported by the two groups quickened the rate at which esters – common chemical groups containing a carbon atom connected to two oxygen atoms and to another carbon atom – normally break apart in water. The increased breakage rate proved the viability of the catalytic antibody concept, notes Kim D. Janda, who works with Lerner at Scripps. But Janda stresses that natural enzyme-driven rates are at least 1 million times faster than the antibody-catalyzed rates achieved in the California studies. Though researchers have throttled up the catalytic rates of some antibodies, they agree that none is quite fast enough yet for large-scale practical applications.

Since 1986, Schultz, Lerner and others have tailored antibodies to catalyze a dozen or so reactions. Combining techniques and principles of chemistry, molecular biology, immunology and genetic engineering, they say they seek to forge a powerful new chemical technology capable of carrying out perhaps millions of reactions.

So far, scientists have designed most catalytic antibodies (called abzymes by some) using the principle of transition-state stabilization formulated by Linus

Pauling. More than 40 years ago, Pauling surmised that enzymes speed reactions by binding reactant molecules into specific transitional shapes, which then quickly rearrange into the final product. Accordingly, Lerner and Schultz reasoned, antibodies that can bind molecules in their transition-state structures should, like enzymes, hasten the reactions.

Putting this principle to work takes diverse expertise. First a chemist makes a stable molecule that closely resembles the fleeting transition state of the reaction the researchers want to catalyze. An immunologist then injects a mouse with the long-lasting transition-state analog, which serves as an antigen. Immune system cells in the mouse's spleen start making antibodies that bind the actual transition state of the reaction. An immunologist removes these antibody-producing cells and individually fuses some of them with modified tumor cells to make hybridomas, or immortal cell lines that continuously churn out lots of identical antibodies — called monoclonal antibodies—which always bind to exactly the same chemical structure. Biochemists then test the monoclonal antibodies of each hybridoma to see which, if any, hasten the reaction.

"If we want to make antibodies that are practical catalysts that can, for example, dissolve blood clots or digest viruses, we need an effective scheme for improving the activity of these molecules," remarked Donald Hilvert, also with Scripps, at an April meeting of the American Chemical Society in Dallas. The best of what Schultz calls the first-generation antibodies fall short of enzymes, which typically pump up a reaction to speeds 100,000 or more times faster. To increase the antibody-catalyzed reaction rates, Schultz says, scientists must learn to combine transition-state stabilization with other tactics within the same second- and later-generation catalytic antibodies.

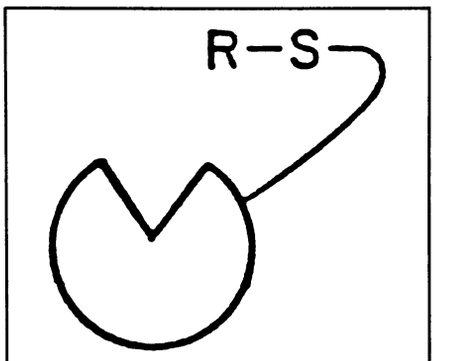
Genetic engineering techniques, innovative chemistry and clever use of the mammalian immune system are beginning to spawn the second generation of faster, more diverse catalytic antibodies, researchers say. With the "bait and switch" tactic, for instance, scientists take an antigen with, say, a positively or negatively charged amino acid (the bait charge) at its binding site, and use it to elicit mouse antibodies with an oppositely charged amino acid (the switched charge) at their binding sites. When such an antibody binds the intended reactant molecules, which might share additional chemical features of the antigen "bait," the charged and strategically located amino acid helps to speed a reaction. The fastest existing second-generation catalytic antibodies remain 1,000 or so times slower than enzymes derived from living cells, but Schultz told SCIENCE NEWS he thinks the two will draw even in a year or two.

Schultz, chemist Stephen J. Benkovic of Pennsylvania State University in University Park and other researchers are using genetic engineering to help them make better catalytic antibodies. Schultz has inserted into bacteria the gene coding for the binding region of an antibody that specifically grabs compounds containing the chemical group 2,4-dinitrophenyl (DNP). With the gene in place in each bacterium, he can use chemical means to trigger a variety of slight mutations in it, resulting in slightly different binding regions. He finds that some of the mutated binding regions actually accelerate reactions with DNP-containing compounds.

Benkovic and his co-workers are trying another tactic. First they gather messenger RNA from antibody-producing spleen cells. Then they develop a library of the antibody genes in bacterial cells, each gene coding for just one of the antibodies elicited by an antigen. In this case, the fluorescent molecule fluorescein serves as a self-revealing antigen. The researchers grow bacterial colonies to generate larger amounts of each antibody, which they then screen for fluorescein-binding ability.

Benkovic says this method should allow his group to screen the binding capabilities of all the antibodies a mouse makes in response to an injection of a particular antigen. This might well supplant the less powerful screening method in which researchers look at only a fraction of the immune response represented by the several dozen or so antibody-producing spleen cells randomly chosen for making monoclonal antibody-producing hybridomas, Benkovic says.

"The objective of this in the long run is to bypass mammalian cells to produce [catalytic] antibodies in bacteria," Benkovic adds. Scientists hope to use such strategies to transform first-generation catalytic antibodies into an improved, faster-paced generation. Another perspective on this experimental tactic, Schultz says, is to view the bacterial system as a means of accelerating the millions of years of evolution that led to the super-efficient enzymes in today's living things.




Schultz/Univ. of Calif., Berkeley

*This Pacman-like object represents a catalytic antibody, and the letter-tipped curve represents a sulfur-containing chemical hook. Chemists can attach a variety of molecules to the "R" site to help catalyze a reaction in the antibody's nearby active site, shown here as the Pacman's mouth.*

Schultz has found another way to add catalytic power to antibodies. He adds a sulfur-containing chemical "hook" or "handle" near the binding site of what he calls semisynthetic catalytic antibodies. The handle itself can serve as a catalytic group, speeding some reactions by a factor of 60,000. More important, Schultz points out, it serves as a generic lure for getting other chemical groups into the binding region of the antibody. In one case, he attached fluorescein to the handle. When the target molecule plugged into the antibody's binding pocket, it quenched the fluorescence. So, by monitoring the dimming of the fluorescence, Schultz could measure how much of the target was present and how fast the antibody bound it.

"Semisynthetic antibodies of this sort

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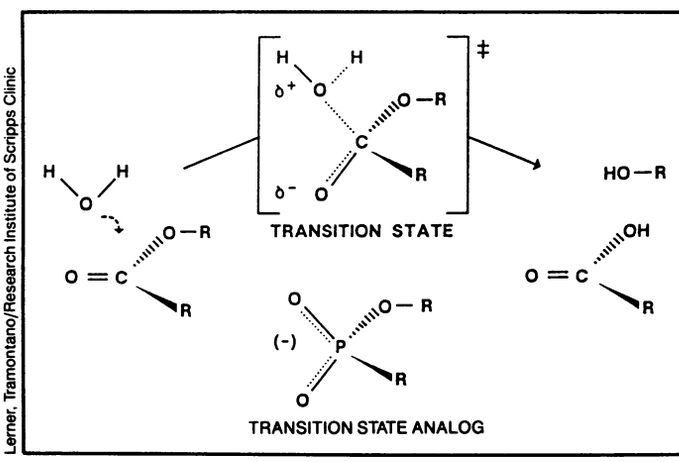


Lerner, Tramontano/Research Institute of Scripps Clinic

*A chemical reaction — the cleavage of an ester molecule by an added water molecule — goes through a fleeting transition state on its way to completion. Synthesizing a stabler, longer-lasting chemical analog of the transition state is the crucial first step in making many catalytic antibodies.*

protective ozone layer rather than repair it, says Gary Whitten, who studies smog ozone at Systems Applications Inc., in San Rafael, Calif.

**Q** *Why not produce pure ozone at the ground and carry it to the stratosphere in airplanes or the space shuttle?*

**A** Several factors, including ozone's explosive nature, keep this idea in the clouds. The paramount problem lies with the energy needed to add ozone to the stratosphere. Total up all the energy humans use today, and it still would not equal the amount needed to reverse the ozone thinning, Rowland says.

As ultraviolet radiation from the sun continually creates vast amounts of stratospheric ozone, other atmospheric processes continually destroy the molecule. The ozone concentrations in the stratosphere therefore represent a balance between these two processes – a situation resembling water flowing into a hole-riddled bucket, says Mark R. Schoeberl, an atmospheric scientist at NASA's Goddard Space Flight Center in Greenbelt, Md.

Theoretically, humans could continually pump up artificially produced ozone molecules in hopes of raising stratospheric levels. Using the water analogy, this would correspond to pouring another stream into bucket. But the artificial stream would not necessarily raise the water level because the laws of physics require that the amount leaking out the bottom must increase as the flow into the bucket grows.

To raise the net water level, humans would have to create an artificial stream nearly as big as the natural stream – in other words, to add almost as much ozone as the sun's energy creates. Such a task would require an energy source that rivals the sun – something clearly out of the human league, Rowland says.

**Q** *How can humans reverse the loss of stratospheric ozone caused by chlorofluorocarbons?*

**A** The only realistic way to raise ozone levels is to plug up some of the holes in the bucket. "That's what we're trying to do by banning [chloro]fluorocarbons," Schoeberl says.

Chlorine levels in the atmosphere have grown significantly since CFCs first reached the market in the 1930s. Today, natural sources such as sea salt contribute only about a third of the chlorine in the stratosphere. The rest comes from CFCs and other industrial chemicals.

Chlorine's destructive power lies in its ability to act as a catalyst. Relatively few chlorine atoms float around in the stratosphere, but a little goes a long way. A single chlorine atom can split 100,000 ozone molecules over the course of a year. Scientists say ozone's only hope lies in lower chlorine levels.

To that end, an international treaty went into effect this year limiting production and consumption of CFCs and related chemicals called halons, which contain destructive bromine. By the end of the century, participating nations must reduce CFC use to half the 1986 levels. But even this will not stabilize the growing chlorine levels in the stratosphere, and many countries now lean toward a full ban on CFCs as early as possible. Other industrial chemicals, such as methyl chloroform and carbon tetrachloride, also release chlorine into the stratosphere, and the Environmental Protection Agency wants to limit these as well (SN: 6/10/89, p.367).

In a sense, humans *can* set the ozone level of the stratosphere, by deciding how much chlorine and bromine to leak into the air. But the protective layer will be a difficult patient to revive. Even if all emissions of these pollutants were to cease today, it would take hundreds of years for chlorine and bromine levels in the stratosphere to return to preindustrial amounts. □

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may prove useful as sensors and diagnostics," he says. He also suggests attaching highly charged metal atoms to the antibodies to enhance some reactions. In another application, Schultz says researchers might hook drug molecules into the handles of antibodies designed to deliver the drug specifically to menacing viruses or cells.

Lerner has made antibodies that have one binding site for a target and another site for hosting a catalytic cofactor. In an example often cited by researchers in this field, Lerner and Scripps co-worker Brent L. Iverson created antibodies that selectively cleave the amide bond between glycine and phenylalanine, two of the 20 amino acids that make up most mammalian proteins. By using a cobalt-containing chemical analog of the glycine-phenylalanine compound as an antigen, the researchers obtained antibodies that bound the amino acid pair along with the metallic (cobalt) cofactor.

Last spring, Janda, Benkovic and Lerner published research results that they say bring catalytic antibodies closer to the toolbox of organic chemists. They reported in the April 28 SCIENCE making a pair of catalytic antibodies, each operating nearly exclusively on one of two mirror-image forms – called enantiomers – of some ester-containing compounds. Quite often, chemists would prefer to work with just one enantiomer. In practice, however, they find it difficult or impossible to separate these otherwise identical chemicals, which often form in equal amounts during chemical reactions. Enantiomer-specific antibodies might enable chemists to routinely focus on just one enantiomer. Other researchers have shown that some catalytic antibodies work even in nonwater, organic solvents, opening the door to their use in a still wider variety of reactions.

Because researchers can make antibodies that bind to virtually any chemical structure, the technology's ultimate applications should reach beyond the horizon of envisioned possibilities, they say. "This technology puts at our fingertips the means to create entirely new enzymes for use as research tools and also in medicine and industry," says Hilvert.

Developing antibodies that catalyze any particular reaction takes a lot of time, money and technical expertise. At least initially, and perhaps for a long time to come, the new chemical tools will find their primary use in the creation of specialized, high-value compounds such as flavor and aroma chemicals, Benkovic and others suggest. In the longer term, Lerner foresees a battery of catalytic antibodies capable of cutting amino acid chains at specific locations that would closely resemble the restriction enzymes that genetic engineers use to specifically snip DNA chains.

Benkovic looks toward pharmaceutical applications. Many drugs come from the manufacturer as an even mix of two enantiomers. Most often, only one enantiomer is medically active, and sometimes the other causes unwanted side effects. Benkovic envisions using custom-designed catalytic antibodies to edit out the unwanted enantiomer, leaving behind a purer drug with fewer side effects.

"Every time we get new antibodies, I kind of look at them like lottery tickets," says Janda of the Scripps Clinic. "When we test them, it's like scratching and seeing if we get rich." Some biotechnology companies already have started catalytic antibody projects in collaboration with university researchers. Schultz says he expects these companies to assemble their own interdisciplinary research teams soon, developing catalytic antibodies independently.

So look out, Raquel Welch. If Hollywood ever decides to remake "Fantastic Voyage," you could end up as a human pretzel. □