Final Report

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GELS FOR MOLECULAR RECOGNITION, ACCUMULATION AND RELEASE

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Principal Investigator

Toyoichi Tanaka
Otto & Jane Morningstar Professor of Science
Massachusetts Institute of Technology
77 Mass Avenue, Cambridge, MA 02139
Tel: (617) 253-4817
Fax: (781) 235-0596
E-mail: toyo@mit.edu
Web site: http://web.mit.edu/physics/tanaka

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Project Summary

Proteins can have the capability of specific molecular recognition and coordination, storage and release. Molecular accumulation by proteins is exceedingly specific and cooperative. The degree of affinity of proteins for specific chemicals can be switched on and off by environmental conditions. One of the most promising yet challenging areas for research in materials science is developing synthetic materials with a capability of specific molecular recognition, accumulation and release similar to that of proteins. These materials could have the accumulative capability of proteins, yet not suffer from the fragility and cost of protein systems. They could be engineered to accommodate substrates which proteins cannot now handle.

We proposed to establish a general principle with which polymer gels can specifically recognize a target molecule and reversibly change their affinity to the target by orders of magnitude. The polymer consists of two species of monomers each having a different role. The majority monomer species control network density and make the gel to reversibly swell and shrink in response to an environmental change such as temperature. The minority monomers come into sufficient proximity to each other when the supporting gel shrinks and then they become able to function as multi-group absorption centers for the target molecules. This absorption can be switched on and off by the reversible gel phase transition. The multiple point absorption is the key not only for reversibility, but also en essential ingredient for possible specificity of target recognition by polymers.

Multivalent metal ions and multi-charged pyranine are used as target molecules to demonstrate the principles, although the method will be applicable to a wide range of target molecules. We believe that he project has indeed establishes and demonstrated the guiding principles for design and synthesis of molecular recognition gels.

We hope that gels which display molecular recognition will find use in a wide variety of applications and cleaning and recovery of chemicals from toxic waste, recovery and concentration of precious molecules from ocean and biological products and catalysis of chemical reactions. They should also shed light on the mechanism of protein functions.

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MPDvorscak May 23, 2001
Mark P. Dvorscak Date
(630) 252-2393
E-mail: mark.dvorscak@ch.doe.gov
Office of Intellectual Property Law
DOE Chicago Operations Office

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SCIENTIFIC ACHIEVEMENTS

We have been fortunate to be able to achieve most of the originally set goals. We summarize the achievement in the following.

1. Reversible molecular absorption based on multiple point interaction by smart gels

In order to demonstrate the general principle of reversible molecular absorption via multiple point interaction, we carefully selected the monomers for the gels and their targets. As target molecules we chose pyranine 3 and pyranine 4 for two reasons. First, they have three or four charges allowing multiple point interactions through the Coulomb force, a simple physical interaction. It was convenient that there are two types of pyranine with different number of charges that allowed us to test the dependence of absorption on the number of contact points. Second their strong fluorescence and clearly separated UV absorption peaks made an accurate determination of the degree of absorption by the gels possible at very low concentrations.
As an absorber we chose methacrylamido-propyl-trimethyl-ammonium-chloride (MAPTAC), that carried one positive charge of the opposite sign to pyranine. Three or four MAPTAC's would capture one pyranine. A small amount of MAPTAC was embedded by copolymerization within a thermo-responsive polymer network of N-isopropylacrylmide (NIPA) in the monomer ratio less than 1/30 (1, 2). In pure water the gels underwent a thermal phase transition at approximately 33°C, swelled below it and shrank above it.
In this project we have been able to discover the power law between the target/gel and the absorbing monomer concentrations. The power corresponds to the number of contact points per target.

$$\text{Affinity} \sim [\text{MAPTAC}]^3 \qquad \text{for pyranine-3}$$

$$\text{Affinity} \sim [\text{MAPTAC}]^4 \qquad \text{for pyranine-4}$$

These power law relationships show that absorption sites are formed when three absorbing molecules (MAPTA) gather to capture one pyranine-3 (three charges) and four MAPTA's are needed to capture pyranine-4 (four charges).
In the swollen state the slope becomes one, indicating that MAPTA absorbs pyranine with a single contact, which is reasonable since MAPTA monomers are well separated from one another. The slope comes back to 3 or 4 upon shrinking indicating their recovery.
In conclusion, we have been able to achieve the reversible multiple point absorption of target molecules. The multiple point interaction was proven by the power law discovered between the affinity and the absorber concentration. This feature is the key for the specificity and reversibility used by protein receptors.

2. Development of gels that can capture and release metal ions.

Gels have been developed that can capture and release multivalent ions including Ca^{++}, Pb^{++}, and Cu^{++} and extensive absorption experiments have been carried out using these gels. In this project we used the similar monomer composition for gels, but as absorption sites, methacrylic acids or acrylic acids were used. It was observed the absorption was high when the gels were collapsed, but dramatically decreased when the gels were swollen. Various absorption curves were obtained using ion-sensitive electrodes specific to each metal ions. The competition between these divalent ions and monovalent ions such as sodium has been studied as a function of their compositions.

3. Finding evidence for molecular imprinting.

One of the challenges we have been struggled for the entire project was to enhance the efficiency of molecular recognition by "imprinting" the target/absorber interaction upon polymerization. In this experiment, we first made complexes between the absorbers and target, then polymerize the gels.
For this purpose we have designed various combinations of absorber, target, solvent, and polymerization conditions. Currently we have been able to observe some imprinting effect in the following systems:

Non-imprinted gels:
lead methacylate (complex of two methacrylic acids and one lead ion)
lead acylate (complex of two acrylic acids and one lead ion)

Non-imprinted gels:
Methacrylate

The imprinted gels are a few times more efficient than non-imprinted gels when the absorber concentrations were lower than that of permanent cross-links. When the amount of absorbing monomers exceeded the permanent cross-link density the difference between the imprinted and non-imprinted gels disappeared.
Molecular imprinting was first demonstrated by Wulff and Mosbach in the late 70's, but was always onto completely rigid plastics. Our experiments showed for the first time that imprinting was possible onto weakly cross-linked gels. This finding demonstrated the possibility to synthesize polymers that can memorize pair wise interaction that was used in proteins. We believe that the result has a direct implication toward better understanding of the protein folding problem.
We have not been successful in observing the imprinting effect on pyranine/MAPTA complex. We have been continuing to break the barrier by testing various polymerization conditions and polymer components.

4. Development of gels that can catalyze chemical reactions.

During the project, we have gone beyond our original plan and have developed gels that catalyze chemical reactions. They may be called artificial enzymes.
Switching on and off of a catalysis is an essential feature of enzymes. Numerous biological activities such as neural signal transmission and muscle contraction crucially depend on the feature. Polymer gels are developed that are capable of catalysis and a catalytic switching. They consist of two species of monomers each having a different role. The major component, N-isopropylacrylamide, controls polymer density and makes the gels to swell and shrink in response to an environmental change such as temperature and solvent. A small amount of imidazole, that binds and hydrolyzes p-phenylnitrile-ester, is copolymerized into the network. Catalytic rate of the gel is low when the gel is swollen. When the gel is shrunken the rate increases by many folds. The switching is discontinuous and reversible and specifically depends on the length of alkyl chains of a substrate. The Michaelis-Menten analysis shows that it is the binding/release of the substrate that causes the switching effect. The gel, when collapsed, develops a highly hydrophobic environment around imidazole attracting the substrate.
The molecular design presented is generic and should be applicable to a variety of catalytic reactions.

POSSIBLE CONSEQUENCES OF RESULTS

Scientific

We believe that the guiding principle has been found for design and development of polymers that are capable of molecular recognition, accumulation and release using simple experimental models. The results of this project sheds light on the mechanism of molecular recognition, accumulation and release by natural proteins, and on design and development of synthetic polymers and gels which can function like proteins in molecular recognition, accumulation and release. The gel we recently developed will help understanding the molecular mechanism of the enzymes.

Technological

The polymer gels may be newly designed and developed and will find applications in various technology fields including:

- Removal and recovery of metals from toxic waste
- Recovery of precious metals from the ocean and other environments
- Segregation, purification and recovery of organic materials in general, including bioproducts
- Sensing and monitoring the presence of target molecules

RECOGNITION AND DEGREES GRANTED

- 1998 Toray Science and Technology Prize was given to Toyoichi Tanaka.

- Ph. D. were given to Rose Du (November, 1998) and Hua Yang (April, 1999)

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