NEW CARBOHYDRATE-BASED MATERIALS

Progress Report
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New Carbohydrate-Based Materials

Abstract

We have prepared a series of new carbohydrate-based materials based on the use of carbohydrates as a template for the introduction of functionality to polymeric materials with complete regio- and stereochemical control. The synthesis of these new materials by the use of chemical and enzymatic methods allows for the rational design of new materials based on the properties of the monomeric subunit. These materials have potential applications that range from their use in enhanced oil recovery to biodegradable plastics to biological applications including targeted drug delivery and enzyme stabilization.

I. Technical Results

A. Overview

Our approach for the preparation of new carbohydrate-based materials is to utilize the carbohydrate as a template for the introduction of desired functionality with complete regio- and stereochemical control by both chemical and enzymatic methods (Scheme I). Attachment of a polymerizable group, for example, by reaction of an aminosugar with methacryloyl chloride, provides a high-yielding method for preparation of a carbohydrate-based monomer. Polymerization using solution or emulsion free-radical methodology gives high molecular weight, water-soluble materials. This approach allows complete control of the composition of the carbohydrate-based polymer: the hydrophilicity, solution viscosity, charge content, and gel formation of the resultant materials are directly related to the functionality contained in the monomer.

Scheme I

We have used these carbohydrate-based polymers, which contain a high-density of masked aldehyde functionality, for coupling with the surface ϵ -lysine residues with of proteins through by reductive amination methodology. This mild, multi-site attachment reaction does not significantly alter the active site of the enzymes or the binding site of antibodies. We have found that the stability of these carbohydrate protein conjugates of proteins [CPC(proteins)] is remarkably high at elevated temperatures in distilled water and buffered solutions and they also retain their catalytic activity in organic solvents.

A project summary for the synthesis and study of new carbohydrate-based materials which was initiated 9 months ago, with the following results: (numbers in parentheses refer the publication list that follows.)

1. **Chemical and enzymatic synthesis of new carbohydrate-based materials:** We have developed a general entry to new carbohydrate-based materials. Our methodology involves the use of a carbohydrate as a template for the introduction of desired functionality with complete stereo- and regiochemical control. These materials are high molecular weight, water-soluble polymers with narrow polydispersities. (3, 4, 5)
2. **Preparation of carbohydrate proteinconjugates:** We have prepared conjugates of these carbohydrate based materials with proteins by the reaction of the latent aldehyde contained in the monomeric subunit of the polymer with ϵ -lysine residues on the surface of proteins. We have prepared conjugates with proteases, antibodies, and an endo nuclease. These conjugates exhibit high stability in aqueous and organic solutions. (3)
3. **Catalytic formation of peptide bonds using carbohydrate proteins conjugates of proteases [CPC(proteases)]:** We have found that CPC(proteases) are stable in organic solvents and efficiently catalyze the formation of peptide bonds. The CPC(proteases) operate on peptide fragments without the need for protecting groups and prior activation. This methodology provides an alternative to solid-phase polypeptide synthesis. (2)
4. **Inhibition of infection with ligands attached to carbohydrate-based materials:** The carbohydrate polymer serves as a template for the attachment of ligands specific for the surfaces of the influenza virus. This approach is expected to be a general approach for the preparation of multivalent materials which will specifically bind to the surfaces of viruses and other organisms.

II. Publications Appearing During This Reporting Period

1. Callstrom, M. R.; Bednarski, M. D. "Chemical and Enzymatic Synthesis of New Carbohydrate-Based Materials" *MRS Bulletin* accepted for publication.
2. Nagy, J. O.; Wang, P.; Gilbert, J. H.; Schaefer, M. E.; Callstrom, M. R.; Bednarski, M. D. "Carbohydrate Materials Bearing Neuraminidase Resistant C-Glycosides of Sialic Acid Strongly Inhibit the *In Vitro* Infectivity of Influenza Virus" submitted for publication.

3. Callstrom, M. R.; Hill, T. G.; Wartchow, C. A.; Huston, M. E.; Smith, M. B.; Bednarski, M. D.; Oehler, L. M.; Wang, P. "The Chemo- and Enzymatic Synthesis of New Carbohydrate-Based Materials and Their Use for the Stabilization of Proteins", In ACS Symposium Series: Washington, D. C. 1992, accepted for publication.
4. Hill, T. G.; Wang, P.; Oehler, L. M.; Huston, M. E.; Wartchow, C. A.; Bednarski, M. D.; Callstrom, M. R. "The Preparation of New Materials for the Stabilization of Proteins." *J. Am. Chem. Soc.* **1992**, *114*, 378.
5. Hill, T. G.; Wang, P.; Oehler, L. M.; Huston, M. E.; Wartchow, C. A.; Bednarski, M. D.; Callstrom, M. R. "Carbohydrate Protein Conjugates (CPC): The Design of New Materials to Stabilize Enzymes." *Tetrahedron Letters* **1991**, *32*, 6823.
6. Wang, P.; Hill, T. G.; Bednarski, M. D.; Callstrom, M. R. "The Use of Carbohydrate Protein Conjugates of Proteases (CPC-Proteases) For The Catalytic Formation of Peptide Bonds." *Tetrahedron Letters* **1991**, *32*, 6827.
7. Hill, T. G., Wang, P., Oehler, L. M., Huston, M. E., Wartchow, C. A., Smith, M. B., Bednarski, M. D., and Callstrom, M. R. "Carbohydrate Protein Conjugates (CPC): The Design of New Materials to Stabilize Enzymes," In *Materials Synthesis Based on Biological Processes*; Alper, M., Calvert, P., Frankel, R., Rieke, P., Tirrell, D., Eds.; *MRS Symp. Ser.* **1990**, *218*, 7.
8. Wang, P., Hill, T. G., Bednarski, M. D., and Callstrom, M. R. "The Use of Carbohydrate Protein Conjugates of Protease [CPC(Proteases)] for the Catalytic Formation of Peptide Bonds," In *Materials Synthesis Based on Biological Processes*; Alper, M., Calvert, P., Frankel, R., Rieke, P., Tirrell, D., Eds.; *MRS Symp. Ser.* **1990**, *218*, 23.
9. Callstrom, M. R.; Bednarski, M. D.; Hill, T. G.; Oehler, L. M.; Gruber, P. "New Carbohydrate-Based Materials," In *Materials Synthesis Utilizing Biological Processes*; P. C. Rieke, P. D. Calvert, M. Alper, M., Eds.; *MRS Symp. Ser.* **1990**, *174*, 259.

III. Research Support (Institute, title, percent effort, annual direct costs, and dates)

A. Matthew R. Callstrom

National Science Foundation "Doped Glassy Carbon Materials (DGC): Their Synthesis and Investigation of Their Properties," \$352,000, Matthew Callstrom and Richard McCreery, co-PI, 9/1/90 - 8/31/93.

Cargill Incorporated "New Carbohydrate-based Polymers," Matthew R. Callstrom, \$350,000, 2/1/89 - 1/31/93.

Department of Energy "New Carbohydrate-based Materials," Matthew R. Callstrom, \$252,000, 9/1/91 - 10/31/94.

OSU Center for Materials Research, "Effects of Absorbed Moisture and Thermal Cycling on the Durability of Polymer Films in Electronic Packaging," \$14,000 (of \$42,000 award with Brian Harper, Peter Anderson, and June K. Lee), 5/1/92-4/30/93.

OSU Center for Materials Research, "New Metal-Carbide Materials for High Temperature Applications," \$25,000 (of \$45,000 award with Patrick Gallagher), 9/1/92-8/30/93.

Amoco Foundation "A Proposal to Amoco Corporation for Support of Equipment and Fellowships in the Department of Chemical Engineering and Chemistry," \$140,000, 11/13/89. Total award to the Department of Chemistry for the purchase of an XPS spectrometer.

IV. Residual Funds

Residual funds will be less than 20% by 11/24/92 which is the end of the first year for this grant.

V. Students Supported by DOE with this Grant

A. *Students completing degrees during 1990:*
none

B. *Students supported during 1990:*

1. Dr. Michael Wheeler, postdoctoral fellow
2. Mr. Charles Wartchow, Ph.D.
3. Mr. Daniel Kahn, undergraduate

VI. Plans for 1991 (Second grant year)

Our plans for the next year include the continued investigation of new carbohydrate-based materials, the properties of these materials and the formation of carbohydrate protein conjugates. Our method of preparation of these materials is unique in both the method and the materials that result. The synthesis of carbohydrate protein conjugates has been quite successful as these materials offer unique opportunities for the preparation of new materials with enzymatic methods.

VII. Appendices (Reprints and Preprints)

These follow in the accompanying pages

*Reprints & preprints
removed & cycled
separately.*

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