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# *Abstract*

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| **Grant Number:** | 5P41RR001614-180063 |
| **PI Name:** | BERTOZZI, CAROLYN R. |
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| **PI Title:** | ASSOCIATE PROFESSOR |
| **Project Title:** | SYNTHESIS OF GLYCOCONJUGATES FOR STUDY OF CARBOHYDRATE PROCESSING ENZYMES |

**Abstract:** Cell surface oligosaccharides play essential roles in the cell-cell recognition events associated with bacterial and viral infection, tumor cell metastasis and leukocyte adhesion at sites of inflammation. These important biological functions obviate importance of studying the enzymes that modulate oligosaccharide structures. We have developed a multidisciplinary program aimed at the study of two oligosaccharide-processing enzymes: carbohydrate sulfotransferases and proximal glycanases. The sulfotransferases have been implicated in the regulation of leukocyte adhesion to endothelium. at sites of inflammation, but have not yet been identified at the molecular level. The proximal glycanases liberate N-linked oligosaccharides from glycoproteins, yet their biological functions remain undefined. Our goals are to identify the mechanisms and biological functions of these enzymes using organic chemistry as a tool. We are synthesizing inhibitors of both classes of enzymes, which will be used to observe the effects of enzyme inhibition on the cellular expression of glycoconjugates. In the case of the sulfotransferases, the inhibitors we synthesize may have anti-inflammatory activity and serve as leads for a new generation of anti-inflammatory drugs. Our synthetic targets comprise complex glycoconjugates related to cell-associated glycoproteins. These molecules are among the most difficult to synthesize and characterize. Mass spectrometry will be an essential tool for the characterization of our synthetic compounds due to their high molecular weight, their structural complexity and their chemical liability. The UCSF Mass Spectrometry Facility is the only facility in the area with stateof-the-art instrumentation and a staff with expertise in glycoconjugate characterization, both of which will be necessary to these projects.

**Thesaurus Terms:**
biomedical resource, endocrine gland /system, hormone, immunology, inflammation, spectrometry, structural biology

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| **Grant Number:** | 1R01GM058867-01 |
| **PI Name:** | BERTOZZI, CAROLYN R. |
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| **PI Title:** | ASSOCIATE PROFESSOR |
| **Project Title:** | METABOLIC ENGINEERING OF REACTIVE GLYCOCONJUGATES |

**Abstract:** Aberrant glycosylation patterns are a hallmark of the tumor phenotype. While highly variable in structure, many tumor-associated oligosaccharides share one important feature: they contain sialic acid residues. Indeed, the overexpression of sialic acid is highly correlated with the malignant phenotype in gastric, colon, pancreatic, liver, lung, prostate and breast cancers, as well as several types of leukemia. Consequently, new strategies for targeting cells on the basis of differential sialic acid expression levels may have widespread utility in the treatment and diagnosis of cancer. This proposal describes a chemicAL approach to the selective targeting of highly sialylated cells with therapeutic and diagnostic agents. The strategy is predicated on the remarkable tolerance of the sialic acid biosynthetic machinery for modified substrates. We have shown that a uniquely reactive functional group, the ketone, can be delivered to cell surface sialic acids by feeding the cells the unnatural metabolic precursor N-levulinoyl mannosamine (ManLev). The ketone provides the ideal mechanism for targeting cells in their native environment because it is chemically orthogonal to all other cell surface components, yet will react selectively with hydroxylamines and hydrazides under physiological conditions. Thus, in the context of the biological milieu, the ketone introduces a unique functional group which permits covalent targeting with molecules bearing complementary functionality. The objective of the proposed research is to explore the potential application of unnatural sialic acid biosynthesis to the selective delivery of therapeutic and diagnostic agents to human tumor cells. A positive correlation between sialic acid expression level and ManLev metabolism is critical for the proposed application, and will be established using tumor cell lines selected for defined sialic acid levels. Next, hydroxylamine-conjugated toxins, imaging reagents and small molecular antigens will be synthesized, and their selectivity for cells rich in sialic acid will be evaluated. As a prelude to future in vivo targeting studies, unnatural sialic acid biosynthesis in laboratory animals will be investigated. Finally, the biosynthetic pathway for cell surface fucosides will be explored as an alternative vehicle for the cell surface delivery of unique chemical targets. This project is the first phase of a long-term program focusing on applications of unnatural oligosaccharide biosynthesis.

**Thesaurus Terms:**

analog, biomarker, chemical structure /function, chemical synthesis, gene expression, glycosylation, neoplasm /cancer diagnosis, sialate
carbohydrate metabolism, chemical conjugate, chemical group, drug delivery system, immunotoxicity, ketone, mannose, neoplasm /cancer chemotherapy
cell line, laboratory rat

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| **Institution:** | UNIVERSITY OF CALIFORNIA BERKELEY<br>BERKELEY, CA 94720 |
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| **Project Start:** | 01-JAN-1999 |
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| **PI Title:** | ASSOCIATE PROFESSOR |
| **Project Title:** | CARBOHYDRATE SULFOTRANSFERASES |

**Abstract:** L-Selectin mediates the initial attachment of blood-borne lymphocytes to endothelial cells during lymphocyte homing to secondary lymphoid organs. In addition, L-selectin participates in the similar process of leukocyte adhesion and extravasation at sites of chronic inflammation. The L-selectin ligands are mucin-like glycoproteins adorned with the unusual sulfated carbohydrate epitopes, 6-sulfo sialyl Lewis x and 6'-sulfo sialyl Lewis x. Sulfation of these epitopes on the N-acetylglucosamine (GlcNAc) and galactose (Gal) residues, respectively, converts inactive glycoforms to high-avidity L-selectin ligands. Furthermore, sulfation of these ligands is restricted in the vasculature to sites of sustained lymphocyte recruitment such as peripheral lymph nodes and chronically inflamed tissues. Therefore, the GlcNAc-6- and Gal-6-sulfotransferases that install the sulfate esters may be key modulators of lymphocyte recruitment to lymph nodes and chronically inflamed tissues, and potential targets for anti-inflammatory therapy. Through a collaborative effort with two other laboratories, three human carbohydrate sulfotransferase clones that may be involved in L- selectin ligand biosynthesis have been identified. The broad objectives of this proposal are the biochemical characterization of these enzymes and the design and synthesis of selective inhibitors. The first Aim of the proposed research is to develop a modular approach to inhibitor design based on the conjugation of two independently-derived compounds, one optimized to bind the carbohydrate binding site and the other optimized to bind the 3'-phosphoadenosine-S'-phosphosulfate (PAPS) binding site. In order to establish a framework for the design of carbohydrate binding site inhibitors, the substrate specificity of each enzyme and the structural features required for recognition will be defined. Preferred carbohydrate substrates will then serve as leads for the design of glycomimetic inhibitors. In parallel, PAPS binding site inhibitors will be identified through the synthesis and screening of aromatic heterocycle libraries. The pharmacophores derived from these parallel efforts will be tethered to produce potent and selective sulfotransferase inhibitors. The second Aim of the proposal is to define the features of carbohydrate sulfotransferase sequences that relate to function by site-directed and domain swapping mutagenesis. The results will contribute to a model for predicting sulfotransferase specificity based on genomic sequence analysis.

**Thesaurus Terms:**
carbohydrate structure, chemical synthesis, enzyme inhibitor, enzyme mechanism, enzyme substrate, oligosaccharide, sulfotransferase
adenosine, enzyme model, enzyme structure, enzyme substrate complex, ligand, protein sequence, selectin, sulfate, sulfation
site directed mutagenesis

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