GLYCOPROTEIN STRUCTURAL BIOLOGY

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Introduction

The central dogma of molecular biology, namely that DNA → RNA → protein, has been a powerful unifying theme in structural biology research over the past decade. However, the central dogma is incomplete because most eukaryotic and many prokaryotic proteins are post-translationally modified. Typical post-translational modifications include acylation by fatty acids, ADP ribosylation, phosphorylation, and ubiquitination, to name a few. The purpose of these modifications is to expand the functionality of proteins by targeting to specific parts of the cell, regulation of activity, or marking for proteolytic degradation. The most prevalent post-translational modification, however, is glycosylation. In fact, more than 50% of all proteins in the human genome contain potential glycosylation sites, more than the fraction of proteins directed to membranes. Biologically, glycosylation has been shown to modify solubility, serum lifetime, aggregation, and enzyme or hormone activity. Glycosylation is the molecular zip code that identifies a particular cell as being of a specific organ or individual. Glycosylation is also important in eukaryotic development.

Despite the biological importance and prevalence of protein glycosylation, less than 2% of all protein structures in the Protein Data Bank are glycoproteins. Furthermore, the majority of these proteins actually lack glycosylation due to bacterial expression or mutational deletion of the glycosylation consensus sequence. Thus, glycoproteins represents one of the few remaining structural biology frontiers. The objective of this research effort is to develop approaches to using high resolution, high-field solution NMR spectroscopy to characterize the structure and dynamics of intact glycosylated proteins. In this project we specifically have investigated methods for isotope enrichment and for enhancing spectral resolution.

Experimental

A major challenge facing structural glycobiologists is the high cost of isotope enrichment in proteins expressed in eukaryotic cells. Metabolism in cultured eukaryotic cells differs considerably from that of bacteria and labeled glucose is rapidly shunted into lactate rather than into energy metabolism or amino acid skeletons. For this reason, glutamine is the major carbon source in culture media. Traditional efforts at isotope enrichment are based on the additional isotopically labeled amino acids obtained from algal cell acid hydrolysate. Acid hydrolysis destroys glutamine and other amino acids, which is added back at considerable expense. Therefore we have initially focused on methods for reducing the dependence on glutamine and increasing the effective utilization of glucose.

Results and Discussion

A 2D $^{1}H,^{13}C$ correlation spectrum of recombinant Thy-1, a 111 amino acid protein containing three asparagine-linked glycosylations expressed in Lec1 mammalian cells [1] grown in serum-free medium supplemented with 4.0 g/L glucose has been obtained. Although we anticipated facile incorporation of labeled carbon into the sugar resonances of Thy-1, we were encouraged to see the extent of amino acids labeled. The labeling pattern was found to be time-dependent. We are currently working to identify the amino acid types that were labeled to provide insights into cellular metabolism in the Lec1 cells, and to provide a possible route towards initial chemical shift assignments.

Conclusions

The work described here is clearly in its preliminary stages. Future work will address additional supplements to the culture medium to extend carbon labeling, and investigating nitrogen labeling.

References

[1] Mehndiratta, P., *et al*., *Protein Expression and Purification*, ***33,*** 274-287 (2004)**.**