Commentary

Glycosylation and the cystic fibrosis transmembrane conductance regulator
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Abstract

The cystic fibrosis transmembrane conductance regulator (CFTR) has been known for the past 11 years to be a membrane glycoprotein with chloride channel activity. Only recently has the glycosylation of CFTR been examined in detail, by O'Riordan et al in Glycobiology. Using cells that overexpress wild-type (wt)CFTR, the presence of polylactosamine was noted on the fully glycosylated form of CFTR. In the present commentary the results of that work are discussed in relation to the glycosylation phenotype of cystic fibrosis (CF), and the cellular localization and processing of ΔF508 CFTR. The significance of the glycosylation will be known when endogenous CFTR from primary human tissue is examined.

Keywords: ΔF508 cystic fibrosis transmembrane conductance regulator (CFTR), oligosaccharides, polylactosamine

Introduction

Interest in glycosylation has been rekindled in the field of CF research since the identification of the CF gene, which encodes the CFTR membrane glycoprotein [1]. The renewed interest has been stimulated by recent developments resulting from attempts to reconcile the proposed function of CFTR with phenomena that are known to be involved in the pathogenesis of the disease. Before the identification of the CFTR gene, many laboratories had described alterations in the glycosylation of CF glycoproteins, but no connection between the altered glycosylation and the pathogenesis of CF was established (for review [2]). This renewed interest fortuitously coincides with the development of automated methods for analysis of the extremely small amounts of relevant oligosaccharides in biologic systems [3].

For the past decade, reports have described CFTR as existing in three different forms, depending on glycosylation: nonglycosylated; core glycosylated; and complex glycosylated, fully mature. It has been reported that only the fully mature form is trafficked to the surface membrane, where it functions as a chloride channel. In some of those studies [4–7] the data have not been completely convincing, although the results have been widely accepted. Nevertheless, the most common mutation ΔF508 has been labeled a processing mutation. It has been reported that, at 26°C, ΔF508 CFTR travels to the surface, where it has chloride channel activity [8]. ΔF508 CFTR is also active when reconstituted into a lipid bilayer [9]. Indeed endogenous ΔF508 CFTR has been identified in the surface membranes of CF cells in culture [10–15]. As yet, no one has directly investigated the carbohydrate structure of CFTR, although many
reports have appeared regarding a glycosylation phenotype of material from CF sources (for review [2]). The CF glyco-
sylation phenotype, which is modulated by CFTR, is
expressed as decreased sialic acid and an increased
amount of fucose linked α1,3 to N-acetyl glucosamine [16].

Oligosaccharides of cystic fibrosis transmembrane conductance regulator
Recently, O’Riordan et al [17] addressed glycosylation of
CFTR directly. Following their earlier studies [18] on the
isolation of CFTR from over-expressing Chinese hamster
ovary and insect cells, CFTR was isolated from several cell
types that were transfected with wtCFTR and that over-
expressed it. The over-expression provided those investiga-
tors with sufficient CFTR to analyze the oligosaccharide
residues in more detail. After immune precipitation the
oligosaccharides were released from CFTR with
N-glycanase and/or endo-β-galactosidase, and were
further separated or examined using polyacrylamide gel
electrophoresis (PAGE) and lectin (Datura stramonium
agglutinin and Maackia amurensis agglutinin) affinity
overlay. N-glycanase releases N-linked oligosaccharides
from the protein, whereas endo-β-galactosidase cleaves
polyglycosaminic containing Galβ1,4GlcNAcβ1,3 repeating
units. They also used fluorophore-assisted carbohydrate
electrophoresis analysis, a highly sensitive method that pro-
vides structural information on purified oligosaccharides.

Interestingly, those investigators found that fully mature,
immunopurified CFTR from Chinese hamster ovary cells
and a mammary tumor cell line (C127) transfected with
wtCFTR contained polyglycosaminic. T-84 cells (a human
colon carcinoma cell line) that were not transfected also
had polyglycosaminic-containing CFTR. The significance
of polyglycosaminic/CFTR would be much greater if endoge-
nous CFTR were extracted from primary human tissue.
Unfortunately, the three cell lines have transformed or
tumor properties, and polyglycosaminic is known to occur in
tumor cells [19] and Chinese hamster ovary cells [20].
Perhaps this is precisely why the authors did not expand on
a relationship to the three size classes of CFTR. In addition,
transfected and tumor cells also have an abundance of tri-
antennary and tetra-antennary oligosaccharides, so this
may have influenced their results [21]. A case in point is the
analysis of CFTR from insect cells, Sf9, which were
infected with wtCFTR/baculovirus. In this case the authors
reported that CFTR contained the insect glycosylation pheno-
type and was not fully glycosylated. As the authors
pointed out, the glycosylation phenotype is influenced by
the host’s enzymes; however, C127 cells transfected with
ΔF508 CFTR showed the PAGE position of core glycosy-
lated CFTR. If confirmed that CFTR isolated from C127
cells after transfaction with ΔF508 was core glycosylated,
then one has to assume that factors in addition to the host
enzymes influence glycosylation of transfected cells.
However, this mutant CFTR was not analyzed.

ΔF508 cystic fibrosis transmembrane conductance regulator
O’Riordan et al [17] pointed out that the elongation of
polyglycosaminic, in the Golgi at 21°C [22], correlates with
the fact that ΔF508 will traffic to the membrane and have
chloride channel activity at a reduced temperature [8].
They suggest that, at lower temperature, ΔF508 arrives at
and traffics through the Golgi at a slow rate and is poly-
glycosaminilated. Another possibility is that an existing
polyglycosaminic is elongated, and the glycosylation
pattern of ΔF508 CFTR must be examined further to differ-
entiate between these mechanisms. In recent studies,
which are yet to be confirmed, a tissue-specific variation of
ΔF508 CFTR expression from null to apparently normal
amounts indicated that ΔF508 CFTR maturation can be
modulated, suggesting that determinants other than CFTR
mislocalization may play a role in ΔF508 CF respiratory
and intestinal disease [23]. Trafficking between the endo-
plasmic reticulum and the Golgi appears more complex
than was originally believed, and it has recently been pro-
posed that wtCFTR follows a unique pathway [24].

In investigations into CFTR in immortalized airway epithelial
cells, Wei et al [10] compared morphologically identifiable
surface membranes with total cell membrane preparations
containing intracellular membranes. Surface membrane
CFTR had lower turnover defined by pulse/chase ratios
than that of the total cell membrane preparations. More-
over, in the presence of 50 μmol/l castanospermine, an
inhibitor of processing α-glucosidases that prevents
binding to calnexin, a more rapid turnover of mutant CFTR
was found in the total cell membrane preparation, whereas
wtCFTR had a slower response. The results are compati-
ble with a pool of CFTR in or near the surface membranes
that has an altered turnover in CF and a glycosylation-
dependent alteration in the processing of mutant CFTR
[10]. It will be interesting to determine whether the surface
membrane population of CFTR molecules is for use exclu-
sively as channel proteins, whereas within the cell more
than one function may be attributed to the protein.

A hypothesis has been proposed [25] that wtCFTR, by
virtue of its proton pump function, contributes to Golgi
processing and sorting [26] in airway epithelial cells. The
hypothesis is supported by recent reports that the relevant
activities of glycosyltransferases and levels of mRNA are
the same in both CF and non-CF airway cells, despite the
differences in the amounts of fucose and sialic acid in ter-
mal positions in the cell surface membrane glycopro-
teins [16,25]. ΔF508 CFTR is proposed to affect
glycoprotein processing in the Golgi, causing faulty com-
partmentalization of the glycosyltransferases, which
results in the CF glycosylation phenotype. That is, if α1,3-
fucosyltransferase is sorted to a compartment positioned
prior to α1,2-fucosyltransferase and sialyl transferase,
then the activity of the latter two enzymes would be
decreased because the same substrate is required for all three enzymes [27]. The glycosylation pattern of wt and mutant CFTR could influence these events.

It has been shown with the use of over-expressing mutants that the features that determine the processing of CFTR are distinct from those that determine channel function [28]. Cunningham et al [29] postulated that the regulatory sites for CFTR trafficking must be either at the trans-Golgi network or peripheral vesicular pools, because the movement of CFTR out of the trans-Golgi network appears to control the onset of CFTR-mediated chloride secretion in a Brefeldin-sensitive pathway [30]. It has been proposed that a difference between CF and non-CF is the inability of ∆F508 CFTR to break out of a recycling pathway between surface membrane and a closely linked compartment [10]. Prince et al [31] reported a lack of internalization of wtCFTR with the addition of cAMP. That hyposialylation occurs in CF cells [10] and that the addition of cAMP increases chloride conductance regulator (CFTR): slow degradation of wild-type and ∆F508 CFTR in surface membrane preparations of immortalized airway epithelial cells. J Cell Physiol 1996, 168: 373-384.

Conclusion
The methods utilized by O’Riordan et al [17] have given the most complete information on the glycosylation of CFTR to date. Those investigators should be congratulated and encouraged to pursue these studies with endogenous human CFTR. The micromethods for oligosaccharide analysis are in place, and both CF and non-CF airway tissues are available. When these analyses are complete, earlier studies can be reassessed and this will provide a more cohesive picture of the relationship of CFTR to glycosylation and of how CFTR is affected by glycosylation.

The key roles of oligosaccharide structures in some disease processes have recently been emphasized [3,33]. As pointed out by O’Riordan et al [17], the glycosylation of CFTR has potential implications for the treatment of CF. The determination of oligosaccharide structures of both mutant and wtCFTR from primary human tissue should provide new insights into the pathogenesis of CF. These new insights will provide the foundation for the development of new therapies for CF.

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