New and Notable

Dynamics of Complex Oligosaccharides

C. Allen Bush
Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland 21228 USA

This issue of the Biophysical Journal features an article by Poppe et al. (1994) that introduces an innovative approach to a problem in the conformation of oligosaccharides about which some controversy has developed over the past decades. One point of view, held by many of the founders of the field (Bock, 1983; Lemieux and Bock, 1983), is that the problem is largely solved and that there exists an adequate theoretical framework employing a "hard sphere" representation of nonbonded interactions. This theory, known as the HSEA method, predicts single rigid conformations dominated by the "exo-anomeric effect." Critics of the rigid school cite NMR (Cumming and Carver, 1987) and fluorescence energy transfer data (Rice et al., 1993), which imply that attempts to find single conformations for oligosaccharides are misguided. Quantitative nuclear Overhauser effect (NOE) measurements and molecular dynamics simulations show that at least some oligosaccharides adopt multiple conformations in exchange (Rutherford et al., 1993; Poppe and vanHalbeek, 1992). Certainly the relatively narrow lines observed in NMR spectra of polysaccharides (2–15 Hz) having molecular masses as high as 100 KDa suggest that there must be some type of flexibility. One possible interpretation is that certain types of oligosaccharides, such as the blood group epitopes, do assume relatively rigid conformations, whereas other sugar linkages are flexible, providing structural "hinges" (Bush, 1992).

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This question of the conformation and flexibility of complex oligosaccharides is one of general biological significance and of specific interest to such a practical field as drug design. If the complex carbohydrates of glycoproteins, glycolipids, and bacterial polysaccharides are to be understood as informational macromolecules controlling interactions at the cell surface, then there must be a mechanism for decoding that information. The class of carbohydrate-binding proteins known as lectins, which have emerged as the primary candidates for this function, has been divided into "S-type" and the "C-type" in a classic review by Drickamer (1988). These proteins have become the target of study by the methods of structural biology as the basis of a new paradigm for drug design (Lasky, 1992).

Specifically, if a flexible ligand (e.g., oligosaccharide) binds to a protein (lectin) in a conformation selected from a manifold of solution conformations, then the freezing of certain of the degrees of freedom of the ligand contributes a negative entropy (and positive free energy) to the binding. Thus, it should be possible to synthesize an analog of the ligand that emulates the selected bound conformation and that could bind much more strongly than the natural ligand. This is a matter of some interest in drug design because the interaction of lectins, such as E-selectin, with monovalent oligosaccharides is often rather weak ($K_d \approx 10^{-4}$ M). Flexibility is also important in the binding of multivalent oligosaccharide ligands that must fold to present several binding epitopes to a multivalent lectin (Rice et al., 1993).

The source of much of the information on conformation of complex oligosaccharides is NOE data, which are quite difficult to interpret for molecules with internal motion. A more direct experimental NMR approach to the problem of flexibility is to measure different relaxation rates, T_1, T_2, $T_{1\rho}$, and NOE of ^{15}N or ^{13}C to sample $J(\omega)$, the spectral density, at a number of different frequencies (Peng and Wagner, 1992). The molecular interpretation of the spectral density is most conveniently done by the method of Lipari and Szabo (1982), in which the types of molecular motion are distinguished by their different time scales. The total motion is separated into a tumbling of the entire molecule (τ_c), which is further modulated by more rapid internal motion with a characteristic time called T_{int} and an amplitude measured by an order parameter, S^2. The separation of the motions into distinct time regimes is crucial to the success of this method. For example, application to methyl group rotation in a slowly tumbling protein is straightforward, but it is not so simple to apply the idea to oligosaccharides where the time scales of the internal motion are not known.

Because most of the published NMR relaxation experiments were not designed with these criteria for time scales in mind, interpretation has been confusing. Yet some of the early NMR relaxation experiments are quite informative. Dill and Allerhand (1979) reported ^{13}C data for glycoproteins that showed that the line widths of the oligosaccharide carbons were much narrower than those of the peptide backbone, suggesting at least a qualitative notion of fast internal motion of the sugars superimposed on slower overall tumbling of the protein. Goux et al. (1982) reported on ovalbumin in which a nonreducing terminal galactose residue was enriched in ^{13}C. Off-resonance rotating frame relaxation data showed that the sugar had a 40- to 80-ps motion superimposed on the slow tumbling of the protein (25 ns). On the other hand, McCain and Markley (1986) reported careful measurements of ^{13}C T_1 of sucrose as a function of magnetic field strength. Their interpretation of a quantitative analysis of the data was that there is rather little internal motion in sucrose. This conclusion has been questioned by Poppe and van Halbeek (1992), who presented a qualitative analysis of ^1H NOE data to show a rigid model for sucrose to be impossible. A

major problem with some of the ^{13}C relaxation studies, especially for small oligosaccharides, is that internal motion may be on a time scale that is close to that of the overall molecular tumbling, making impossible their separation on the basis of time scales.

A possible solution to this problem is proposed by Poppe et al. (1994), who report ^{13}C T_1, $T_{1\rho}$, and NOE for the complex ganglioside GD1a, inserted in a micelle. The slow tumbling of the micelle provides the distinct time scale, revealing the relatively faster internal motion of a flexible glycosidic linkage in the glycolipid. The micelle plays a role analogous to that of the protein in the glycoprotein data discussed above, and the results are comparable. There are many technical difficulties in working with a complicated assembly such as a glycolipid in a micelle, but careful studies of the type reported by Poppe et al. (1994) could instruct us not only about oligosaccharide flexibility but also about how glycolipids move in membranes.

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How ATP Regulates the CFT Regulator

Louis J. DeFelice

Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322 USA

For more than ten years we have known that cystic fibrous (CF) involves a defect in Cl conductance. Numerous articles have appeared that elucidate the mechanisms of this defect. Yet the obvious question remains: how does CFTR normally work. Michal Winter et al. (1994, this issue) make a significant step toward understanding that question by studying the ATP regulation of CFTR Cl channels. The cystic fibrous transmembrane conductance regulator (CFTR) has a curious name because the gene, cloned five years ago, was neither clearly a channel nor a channel regulator. We now know that the CFTR is a moderately low-conductance ohmic channel that selects Cl over other ions and responds to cAMP-dependent protein kinase A (PKA) and ATP. Viewed as a channel, CFTR seems fairly ordinary. What elevates it is that CFTR malfunctions in patients with CF. If the CFTR protein (~1500 residues) lacks one phenylalanine at position 508, it fails to reach the apical membrane of the airway epithelium. This most common naturally occurring mutation is responsible for 70% of all CF cases (~35,000 in the U.S. alone). In a rarer form of the disease, CFTR mutants get to the right place but fail to function. Thus, either the absence of these Cl channels or the collapse of Cl pathway can cause CF. The strategic location of the CFTR in the pulmonary epithelium and its probable role in the co-transport of water are the primary reasons. The airways of CF patients have abnormally thick mucus, and dehydration is the major problem. Because the movement of water requires the movement of Cl, problems with CFTR manifest as debilitating congestion.

Welsh and Anderson were among the first to show that low temperatures allow the F508 mutants to reach the cell surface. This discovery resolved several conflicting reports. Once they were in place, the F508 mutants can conduce Cl. This result helped reduce CF to a problem in protein trafficking—now under intense investigation. However, a substantial fraction of CF cases involves CFTRs that express normally do not transport Cl. Perhaps we could understand why the channel does not conduct Cl if we clearly understood normal conduction. The breakdown of function may reduce to the question: what regulates the CFT regulator?

We already know that CFTR must bind ATP. However, it remains unclear whether CFTR must also hydrolyze ATP. In one recent model, ATP activation occurs in two steps. ATP binds to two membrane domains, and then PKA phosphorylates a cytoplasmic loop called the R domain (Anderson et al., 1991). Despite numerous efforts, we still have very little understanding of how ATP interacts with these nucleotide-binding regions. Presumably, the R domain plugs the pore that conducts Cl. Perhaps phosphorylation adds negative charge to the R domain, leading to conformational rearrangements that open the channel (Rich et al., 1993). Numerous questions remain. If R is the plug that closes the channel, what exactly does phosphorylation do?

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