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Glycobiology Conference 2017: Comparison of different approaches for quantitative N-, O-linked glycan and monosaccharide composition analysis in biopharmaceutical production - Iva Turyan - Biogen

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Glycosylation of therapeutic recombinant proteins is of importance due to its potential impact on solubility, bioactivity, pharmacokinetics and immunogenicity of glycoprotein pharmaceuticals. Detailed characterization of glycans present on recombinant glycoprotein remains an important challenge in the development and production of biotherapeutics. Analytical strategies for characterization of N- and O- glycosylation and monosaccharides analysis will be presented. These include comparison of HILIC-FLR, MALDI-TOF MS and CE-LIF for N-glycan analysis, choice of a method for quantitative and non-selective release of O-linked glycans, and selection of a method for monosaccharide composition analysis. With few exceptions (e.g., deoxyribose), monosaccharides have this chemical formula: (CH2O)x, where conventionally x ≥ 3. Monosaccharides are often classified by the amount x of carbon atoms they contain: triose (3), tetrose (4), pentose (5), hexose (6), heptose (7), and so on. Glucose, used as an energy source and for the synthesis of starch, glycogen and cellulose, may be a hexose. Ribose and deoxyribose (in RNA and DNA respectively) are pentose sugars. Samples of heptoses include the ketoses, mannoheptulose and sedoheptulose. Monosaccharides with eight or more carbons are rarely observed as they're quite unstable. In aqueous solutions monosaccharides exist as rings if they need quite four carbons. Two monosaccharides with equivalent molecular graphs (same chain length and same carbonyl position) should be distinct stereoisomers, whose molecules differ in spatial orientation. This happens as long as the molecule contains a stereogenic center, specifically an atom that's chiral (connected to four distinct molecular sub-structures). Those four bonds can have any of two configurations in space distinguished by their handedness. During a simple open-chain monosaccharide, every carbon is chiral except the primary and therefore the last atoms of the chain, and (in ketoses) the carbon with the keto group. For instance, the triketose H(CHOH)(C=O)(CHOH)H (glycerone, dihydroxyacetone) has no stereogenic center, and thus exists as one stereoisomer. the opposite triose, the aldose H(C=O)(CHOH)2H (glyceraldehyde), has one chiral carbon — the central one, number 2 — which is bonded to groups −H, −OH, −C(OH)H2, and −(C=O)H. Therefore, it exists as two stereoisomers whose molecules are mirror images of every other (like a left and a right glove). Monosaccharides with four or more carbons may contain multiple chiral carbons, in order that they typically have quite two stereoisomers. The amount of distinct stereoisomers with an equivalent diagram is bounded by 2c, where c is that the total number of chiral carbons. The Fischer projection may be a systematic way of drawing the skeletal formula of an acyclic monosaccharide in order that the handedness of every chiral carbon is well specified. Each stereoisomer of an easy open-chain monosaccharide are often identified by the positions (right or left) within the Fischer diagram of the chiral hydroxyls (the hydroxyls attached to the chiral carbons). Most stereoisomers are themselves chiral (distinct from their mirror images). Within the Fischer projection, two mirror-image isomers differ by having the positions of all chiral hydroxyls reversed right-to-left. Mirror-image isomers are chemically identical in non-

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chiral environments, but usually have very different biochemical properties and occurrences in nature. While most stereoisomers are often arranged in pairs of mirror-image forms, there are some non-chiral stereoisomers that are just like their mirror images, in spite of getting chiral centers. This happens whenever the molecular graph is symmetrical, as within the 3-ketopentoses H(CHOH)2(CO)(CHOH)2H, and therefore the two halves are mirror images of every other. Therein case, mirroring is like a half-turn rotation. For this reason, there are only three distinct 3-ketopentose stereoisomers, albeit the molecule has two chiral carbons. Distinct stereoisomers that aren't mirror-images of every other usually have different chemical properties, even in non-chiral environments. Therefore, each mirror pair and every non-chiral stereoisomer could also be given a selected monosaccharide name. For instance, there are 16 distinct aldohexose stereoisomers, but the name "glucose" means a selected pair of mirror-image aldohexoses. Within the Fischer projection, one among the 2 glucose isomers has the hydroxyl at left on C3, and at right C4 and C5; while the opposite isomer has the reversed pattern. These Specific monosaccharide names have conventional three-letter abbreviations, like "Glu" for glucose and "Thr" for threose. Generally, a monosaccharide with an asymmetrical carbons has 2n stereoisomers. The amount of chain stereoisomers for an aldose monosaccharide is larger by one than that of a ketose monosaccharide of an equivalent length. Every ketose will have 2(n−3) stereoisomers where n > 2 is that the number of carbons. Every aldose will have 2(n−2) stereoisomers where n > 2 is that the number of carbons. These also are mentioned as epimers which have the various arrangement of −OH and −H groups at the asymmetric or chiral carbon atoms (this doesn't apply to those carbons having the carbonyl functional group). Like many chiral molecules, the 2 stereoisomers of glyceraldehyde will gradually rotate the polarization direction of linearly polarized light because it passes through it, even in solution. The 2 stereoisomers are identified with the prefixes d- and l-, consistent with the sense of rotation: d-glyceraldehyde is dextrorotatory (rotates the polarization axis clockwise), while l-glyceraldehyde is levorotatory (rotates it counterclockwise). The d- and l- prefixes also are used with other monosaccharides, to differentiate two particular stereoisomers that are mirror-images of every other. For this purpose, one considers the chiral carbon that's furthest far away from the C=O group. Its four bonds must hook up with −H, −OH, −C(OH)H, and therefore the remainder of the molecule. If the molecule are often rotated in space in order that the directions of these four groups match those of the analog groups in d-glyceraldehyde's C2, then the isomer receives the d- prefix. Otherwise, it receives the l- prefix. Within the Fischer projection, the d- and l- prefixes specifies the configuration at the atom that's second from bottom: d- if the hydroxyl is on the proper side, and l- if it's on the left side. Note that the d- and l- prefixes don't indicate the direction of rotation of polarized light, which may be a combined effect of the arrangement in the least chiral centers. However, the 2 enantiomers will always rotate the sunshine in opposite directions, by an equivalent amount. cf. d/l system. In this seminar, I will discuss appropriate glycoanalysis methods which allowed detecting changes in glycosylation parameters. A case study will be presented that highlights glycoanalysis techniques useful for gaining understanding of the relationship between process inputs (raw materials) and product quality attributes. The findings confirm that the glycosylation profile of therapeutic antibodies needs to be monitored through development to ensure consistency, efficacy, and safety of therapeutic products.

Biography

Iva Turyan has her expertise in characterization of N- and O-glycosylation and monosaccharide analysis at the level of released glycans and intact therapeutic recombinant protein. She has

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completed her PhD from St. Petersburg University and Postdoctoral studies at The Hebrew University of Jerusalem. She is currently an Analytical Development Scientist at Biogen, Cambridge, MA. She has published more than 45 papers in reputed journals, and has been awarded 6 patents.

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