Attending the Tenth International Symposium
on Glycoconjugates
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From September 10th to 15th, 1989 the 10th International Symposium on Glycoconjugates was held in Jerusalem, Israel. The symposium was organized by Professor N. Sharon of Weizmann Institute and more than 400 researchers from about 25 countries participated. The prestigious Ramada Renaissance Hotel was selected for the meeting and enthusiastic discussions were held in a placid and refined atmosphere. The size of the meeting place was carefully selected in view of the number of attendants, and meeting places for glycoprotein, glycolipid, and posters were compactly arranged and inter connected, not requiring from the attendants extra time to move from one place to the other.

The symposium was intended for highly professional researchers who speak the common language of saccharide and every theme was so interesting. Because all were related to our studies we had quite a hard time trying not to miss anything.

Considering that the organism is one system and life is a complex made of various elements acting on one another, and that a change of individual elements has a great effect on the overall system, diversity of sugar chains of complex

carbohydrates, which for some researchers seems to be a bog from which they hardly can find a way out, has an important meaning that is directly related to mutual recognition, which is indeed an essence of life, such as a specific difference or organic difference that cannot be explained merely by the change of protein structure, nonuniformity of intracellular distribution, and time change in the process of development and differentiation. This paper outlines the symposium with special emphasis on the themes which attracted our attention.

The lecture on the first day titled topobiology was started by G.E. Edelman (Rockefeller University). Possibly due to massive contents, the portion directly related to the sugar chain was comparatively little discussed. He advanced a hypothesis that a negative charge of sialic acid of the sugar chain of cell adhesion molecule (CAM) existing on the cell surface is useful to stick the hydrophobic portion of a molecule out of the cell, and this stuck out portion takes part in intercellular recognition. The group consisting of Roth et al. (Basel University) reported on N-CAM in the poster session that highly sialylated molecules are observed in various human tumors.

What attracted people's attention in the subsequent session treating the structure and functions of glycoprotein sugar chain was the report of J.U. Baenziger (Washington University). The LH and FSH of pituitary hormone consist of a common α subunit and a hormone-specific β subunit and a conspicuous difference is recognized in the sugar chain. In particular, the β 1,4-bonded GalNAc residue of the LH molecule is a very unique existence among N-glycoside type glycoproteins. Here, they scrutinized receptor specificity of this transferase and concluded that it recognized peptide as a common α subunit, LH β , and native LH molecule, and

proved that it exhibited an activity in the transfer of GalNAc residue to GlcNAc₂Man₃GlcNAc₂. They also supposed that this sugar was not transferred to FSH, for FSH β masks the recognizing portion of the α subunit. It has been considered that sugar chains of the α subunit have some effects on the manifestation of the functions of this hormone in the target cell, and in spite of the fact that both hormones are synthesized in the same cell, the secreting process is controlled completely separately, indicating that these sugar chains have an important meaning in hormone secretion control as well as in the sulfation of this GalNAc residue.

On the other hand, for the diseases involving sugar chains as important factors, Kobata (University of Tokyo) made a report on the IgG sugar chains of patients with Rheumatoid arthritis (RA). That is, he explained that the terminal galactose content of N-glycoside type sugar chains bonded to FC portions of IgG of an RA patient decreased since no such decrease was observed in sugar chains of serum transferrin obtained from the patient, it was regarded as some kind of defect in the synthesizing process. It has already been proved in the molecular structure level that this Gal residue interacts strongly with the protein core of the IgG molecule, and that this terminal sugar cannot be cut off with ordinary β -galactosidase. Kobata and his group separated an enzyme acting on this sugar chain from bacteria to prepare agalactoIgG, and clarified the decrease in galactosyltransferase activity of patient's B cell using agalactoIgG as a receptor. Of course, many other possibilities must be studied, but their theory attracted attention particularly because it might serve in elucidating the causes of incurable diseases. Questions were raised about sugar chains bonded to the variable region, and there still

remains a wide field of study on sugar chains of immunoglobulin.

As mentioned in the report of Baenziger, it is clear that specificity of the receptor of glycosyltransferase is stricter than we thought and depends not only on the sugar chain structure but also on the stereostructure of receptor protein. It shows that the use of a native receptor is indispensable for simulating reactions in vivo.

It is the glycosyltransferase that expresses information from a gene as a sugar chain structure, and due to its importance as a contact point of information transmission, many reports were made on glycosyltransferase in this symposium. Applications of genetic engineering techniques are now planned for enzymes related to blood types, sialyltransferases, and transferases related to synthesis of N-glycoside type sugar chains.

For biosynthesis of N-glycoside type sugar chains, R.G. Spiro (Harvard Medical School) reported a hydrolysis activity of oligosaccharide transferase, which is thought to be related to the control of oligosaccharide lipid pool. They found a new endo-mannosidase which exists in the Golgi apparatus and cuts out the glucose-substituted mannose residue and proved the existence of a new bypass in the conventional biosynthesis process. What was impressive was a beautiful TLC spot. According to Dr. Goso (Kitasato University) who once studied in the Spiro's laboratory, considerable efforts (or failures?) and concentration were required to prepare a chromatogram of that quality.

Kornfeld (Washington University) investigated conditions in which phosphorylation takes place in the Man residue of sugar chains (as a result it moves to ER, Golgi, then Lysosome) using a genetic engineering technique. They could not find common amino acid sequences that may possibly

cause phosphorylation in the Man residue from as many as 50 lysosome enzymes. They made chimeras of the enzyme which was secreted after synthesis and lysosome enzyme, and investigated which parts of the lysosome enzyme were important. As the secreted enzyme, they selected pepsinogen which is not a glycoprotein, and using cathepsin D with two sugar chains as a lysosome enzyme they varied the length in many ways to prepare a chimera between the two enzymes, and investigated whether it moved to lysosome. As a result, with 188 - 230 and 265 - 319 portions of cathepsin D, phosphorylation occurred in Man of two sugar chains of the chimera enzyme. Furthermore it was shown that Lys²⁰³ in the arrangement is decisively important. However, it is said that cathepsin itself forms Man-6-P even if Lys²⁰³ is substituted with other amino acids. It seems that the time when the genetic engineering technique exercises great influence over sugar chains not subject to direct control of DNA has already arrived.

Various developments of sugar chain analysis techniques indispensable for studies of sugar chain structures and functions are now under way. For separation, in the session sponsored by Dionex Lab. the oligosaccharide mapping using Pulsed Amperometric Detection Method (PAD) was reported. It was also shown that by the same method neutralizing the alkaline eluate as soon as it is eluted allows for its application in separating to prepare oligosaccharide.

Various technologies with new possibilities, such as combination of HPLC and MS analysis by liquefied carbon dioxide gas were introduced, though they seemed to be still unknown quantities. In the poster session, a high sensitive detection technique combining digoxigenin, lectin, and immunoprocess was reported by Haselbeck of Boehringer while the analysis of O-glycoside type sugar chains using

neoglycolipid was reported by Stoll et al. (MRC Clinical Research Center), and two-dimensional mapping of pyridyl aminated oligosaccharide was reported by Takahashi et al. (Nagoya City University).

Together with these sugar chain separating techniques, as mentioned in the lecture given by Vliegenthart (Utrecht University) on the second day and in many posters, the NMR analysis has now become an indispensable means for the analysis of sugar chain structures. Perhaps in the years to come, sugar chain structure analysis will be automated as with amino acid or amino acid sequence analysis, allowing even non-specialists in sugars to analyze them easily.

Studies on the sugar chain stereostructure by the full use of computers are now under way, and interaction between protein and sugar, for example, contributions of sugar to stereostructure of protein, and the role of protein functions as a modulator will be elucidated by such frontier researches.

In addition, W. J. Whelan (Miami University) proved that biosynthesis of muscle glycogen begins with the formation of autocatalytic glycogen primer on the tyrosine residue of glycogenin, providing a solution of the problems that have remained unsolved. G. W. Hart et al. (Johns Hopkins University) investigated intracellular distribution of protein with O-glycoside-linked GlcNAc, and proved that this O-GlcNAc glycoprotein is localized in a nucleus and cytoplasm. The salivary chromosome of a vinegar fly is strongly bonded to this glycoprotein and FITC-WGA staining reveals a characteristic striped pattern, and the stainability decreases at the puffing regions in which m-RNA is synthesized actively. The O-GlcNAc level is high in the DNA synthesis preparation period (G1 period) and decreases

rapidly during DNA synthesis period (S period) and cell division (G2/M period).

Regarding the glycolipid discussed in the general lecture, their effects contributed externally to cell differentiation and functions and were popularly taken up, but among them the greatest work was reported by Hakomori.

Hakomori (University of Washington) indicated that glycolipid has a biomodal function; functional modulator and cell social interaction. His team has presented so many examples of modulators related to growth hormone receptor. It was added that, insulin receptor phosphorylation is inhibited by 2-3SPG (2-6SPG does not work). However, the highlight was a carbohydrate-carbohydrate interaction.

By now they have shown that there is a reaction between Le^X (see JBC 264 (1989) 9476 - 9484). In this symposium, they reported recognition reaction between hetero saccharide chains which excited all the attendants. The GM3-rich B16 melanoma can be attached to mouse lymphoma L5178AA (Gg3 = Asialo GM₂). On the other hand, it is not attached to mutant AV27 B16 melanoma which does not have Gg₃. That is, does GM₃ react with Gg₃? Hakomori proved in detail the interaction of GM₃ and Gg₃ by showing, for example, adsorption experiments of cells to glycolipid-coated plastic and the interaction of liposome containing glycolipid with other glycolipid coated on the plastics, in the same manner as he showed the interaction between Le^X - Le^X in the previous report in JBC. In this event, Ca^{++} and Mg^{++} are required. Liposome GM₃ and liposome Gg₃ showed an affinity of $K_d = 5.9 \times 10^{-6} \text{M}$.

Why do sugar chains react with each other in a specific manner? When the oligosaccharide model is assembled into a molecular model in such a manner as to minimize free energy, hydrogen collects at one end to become hydrophobic, whereas

the hydroxyl group collects at the other end and becomes hydrophilic. This principle applies to all Le^x , GM_3 , and Gg_3 . Interaction between proteins also takes place at the hydrophobic site, and therefore the sugar may be bonded between hydrophobic site. Hakomori emphasized that the recognition reaction between sugar chains is the first step of the intracellular interaction. Of course, he did not deny the existence of reactions between sugar and lectin and integrin and cell adhesion molecules, but only emphasized the possibility of the first reaction. Le^x exists not only in glycolipid but also in glycoprotein, but GM_3 and Gg_3 are exclusive members in glycolipids. Consequently, when the cells approach, they react with each other over protein, proteoglycan, and intracellular substance located between cells or on the cell, but is it really possible?

To mention some of the impressions we had besides the lectures, maybe because many women majored in this field of study, many woman researchers were appointed as chairpersons, creating a very mild atmosphere. I (Iwase) had a sad experience in taking photographs of posters which when developed showed that flashlight had entered the film or fine-printed data were not legible. There might be a problem of copyright, but there were some people distributing poster copies at the meeting place. I think this is a very good idea.

Finally, I (Iwase) joined the preconference tour held by Kenes Tour and had a wonderful time in Elath facing the Red Sea. Even the harsh examination at the Tel Aviv Airport would become a good memory with the word "For you" from a beautiful officer.