of the cell surface materials were explored in detail using the ciliary epithelium of an albino rabbit.

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LECTIN-SEPHAROSE COLUMNS AS NEW TOOLS FOR THE FRACTIONATION OF GLYCOPEPTIDES

AKIRA KOBATA, TAKASHI MURAMATSU, SHUN-ICHIRO OGATA
AND SEIICHI TAKASAKI

Department of Biochemistry, Kobe University School of Medicine, Kobe 650

Lectin is a general term given to proteins or glycoproteins which are derived from several plant sources and have ability to bind to sugar residues of specific structure. By making use of the specificities, they have been applied to elucidate the changes and functions of cell surface glycoconjugates in various physiological states. Like the reaction between antigen and antibody, affinity of lectin to sugar residues varies with the slight differences in the structures of sugar residues. With this characteristics, lectin can be used as a new tool for the fractionation of sugar

chains. Actually, several glycoproteins have been purified by affinity column chromatography on concanavalin A-Sepharose. However, concanavalin A-Sepharose affinity column chromatography could not become a really effective tool for analysis of sugar chains without elucidating the relation between the structures of sugar chains and their behaviors on the column.

In order to clarify the relation, we have examined the behaviors of several oligosaccharides and glycopeptides of known structures on the column and have obtained the following rules. As already reported by Goldstein *et al.*, sugar residues reactive with concanavalin A-Sepharose column are α -mannopyranosyl and α -glucopyranosyl residues of which all the hydroxyl groups except C-2 position are free. Oligosaccharides or glycopeptides which contain only one such reactive residue in one molecule, however, cannot be bound to the column. Occurrence of more than two reactive sugar residues in one molecule are necessary for them to be bound to the column. The binding force is proportional to the number of reactive residues in a molecule. Thus, glycopeptides from thyroglobulin unit A which has several Man α 12 Man branches are tightly bound to the column and cannot be eluted from the column by α -methylmannoside.

Glycoproteins of cell surface membrane contain a number of α -mannosyl residues. Therefore, concanavalin A-Sepharose column could be a useful tool for the fractionation of cell material and for detection of structural changes in sugar chains induced by the alterations of physiological condition of cells. In fact, fractions of glycopeptides which were purified from fibroblast and considered to be homogeneous by conventional criteria, *i.e.*, electrophoresis and gel filtration, have further been separated into two fractions respectively by concanavalin A-Sepharose column. This result shows the usefulness of the column as a new tool for the fractionation of sugar chains.

The above mentioned principle of fractionation by concanavalin A-Sepharose column has been deduced from behavior of single sugar chain on the column. But a similar principle seemed to occur in case of glycoprotein which has many sugar chains in one molecule. For instance, lectin derived from *Phaseolus limensis* (lima bean) reacts with α -N-acetylgalactosamine residue. Column of this lectin conjugated to Sepharose can bind blood group A active mucin. However, if the sugar chains are released from the polypeptide core of the mucin by mild alkaline hydrolysis, they can no longer be bound to the column although the released sugar chains still keep their original structures reactive with the lectin. This fact indicates that the occurrence of multiple reactive groupings in one molecule is necessary for the mucin to be bound to the column.

Although affinity column chromatography on lectin-Sepharose is quite useful, attention must be paid in interpreting the results of lectin-Sepharose column chromatography analysis, since the material which shows no interaction does not necessarily lack the sugar chain reactive with the lectin.