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Radioactive fucose as a tool for studying glycoprotein secretion

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Abstract

The efficiency and reliability of radioactive fucose as a specific label for newly synthesized glycoproteins were investigated. Young adult male rabbits were injected intravitreally with [^{3}H]-fucose, [^{3}H]-galactose, [^{3}H]-mannose, N-acetyl-[^{3}H]-glucosamine or N-acetyl-[^{3}H]-mannosamine, and killed 40 h after injection. In another series of experiments rabbits were injected with either [^{3}H]-fucose or several tritiated amino acids and the specific activity of the vitreous proteins was determined. Vitreous samples were also processed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and histological sections of retina, ciliary body and lens (the eye components around the vitreous body) were processed for radioautography. The specific activity (counts per minute per microgram of protein) of the glycoproteins labeled with [^{3}H]-fucose was always much higher than that of the proteins labeled with any of the other monosaccharides or any of the amino acids. There was a good correlation between the specific activity of the proteins labeled by any of the above precursors and the density of the vitreous protein bands detected by fluorography. This was also true for the silver grain density on the radioautographs of the histological sections of retina, ciliary body and lens. The contribution of radioautography (after [^{3}H]-fucose administration) to the elucidation of the biogenesis of lysosomal and membrane glycoproteins and to the determination of the intracellular process of protein secretion was reviewed. Radioactive fucose is the precursor of choice for studying glycoprotein secretion because it is specific, efficient and practical for this purpose.

Key words
- Glycoprotein
- Fucose
- Radioautography
- Secretion
- Vitreous body

Introduction

Glycoproteins are characterized by carbohydrate side chains covalently bound to the polypeptide backbone (1,2). While the polypeptide chains are unbranched polymers, the carbohydrates form branched oligosaccharides and their variable types of bonds lead to the existence of numerous isomers (3,4). Glycoproteins are ubiquitous compounds. Indeed the overwhelming majority of plasma proteins (with the exception of albumin) are glycoproteins. Some hormones or pro-hormones are glycoproteins: FSH, LH, TSH and pro-ACTH/MSH in the pars distalis of the pituitary gland, pro-vasopressin in the hypothalamo-neurohypophyseal system, thyroglobulin, and others. All the antibodies and almost all lysosomal hydrolases are glycoproteins, and other enzymes such as ribo-

nuclease and some membrane-bound ones are also glycoproteins. Structural proteins such as collagens and all the components of the basement membrane are glycoproteins (5). These compounds are also found in exocrine secretion, the most common being the salivary mucins. Numerous glycoproteins have been characterized as integral components of the plasma membrane where they play several roles (1,2).

The carbohydrate portion of glycoproteins may account for as little as 1% or as much as 80% or more of their molecular weight (1,2). The functions of the sugars in glycoproteins are still controversial. In some cases, it is clear that they protect the molecule against the proteolytic action of some enzymes (this has been shown for ribonucleases) while in other instances it is hard to assign any specific role to the sugars (6-9).

Although numerous monosaccharides (perhaps more than 200) have been characterized in nature, only nine of them were identified in glycoproteins (3,4): D-glucose (Glc), D-galactose (Gal), D-mannose (Man), L-fucose (Fuc), N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc), sialic acids (Sa), L-arabinose (Ara) and D-xylose (Xyl).

The types of bonds between the nine monosaccharides and the twenty amino acids involved in protein synthesis are also limited to a few: GlcNAc-asparagine, GalNAc-threonine (serine), Xyl-serine, Gal-hydroxylysine and Ara-hydroxyproline are the most prevalent (3).

The arrangements of the monosaccharides in the side chains may be quite variable but some almost invariable situations occur. For example, when the bond between the oligosaccharide and the polypeptide chain is GlcNAc-asparagine, mannose is invariably a component of the side chains. On the other hand, mannose is not present when the bond is of the GalNAc-threonine type (serine). Peculiar types of side chains cause proteoglycans to be particular types of glycoproteins. Thus, with the exception of glycogen and hyaluronic acid, all carbohydrate polymers in the animal kingdom are linked to proteins, and, to a lesser extent, to lipids (10,11).

The biosynthesis of glycoproteins has been the subject of several reviews (12-17) and will not be described here. The main purpose of the present study is to show the reliability, feasibility and convenience of the use of radioactive fucose for the investigations on glycoprotein synthesis and secretion.

Among the above mentioned nine sugars, sialic acid cannot be used as a precursor because it does not penetrate the cells as such. Radioactive N-acetyl-mannosamine is the precursor of choice for the studies on sialic acid incorporation into glycoproteins. However, it shows a poor efficiency for high resolution radioautography since it has been demonstrated that a massive dose was required to obtain a light reaction at short time intervals after its intravenous injection (18). The monosaccharides more often employed in radioautographic studies of glycoprotein synthesis are fucose, galactose, N-acetyl-glucosamine, mannose and N-acetyl-mannosamine. To our knowledge, there is no study in which [^{3}H]-mannose was intravenously or intraperitoneally injected and the tissues were then processed for radioautography. A few investigations using [^{3}H]-mannose were performed with tissues kept in culture on Petri dishes (19,20). *In vivo* studies with [^{3}H]-galactose were carried out but it was necessary to employ doses of the order of 125 µCi/g body weight to obtain some incorporation after its intravenous injection (21,22). The main disadvantage of mannose and galactose is their quick conversion into glucose after systemic administration, as demonstrated by Leblond and co-workers at McGill University. This does not happen so often with N-acetyl-glucosamine. Most of the above sugars are not found free in the body. Instead, they are conjugated with nucle-

otides and this is a requirement for their incorporation into glycoproteins because the enzymes performing this function recognize the sugar only when it is conjugated with a specific nucleotide. Therefore, the incorporation of [^{3}H]-mannose, for example, could be hampered not only by its conversion into glucose but also by the existence of a large pool of natural GDP-mannose. By the way, endogenous GDP-mannose may be converted into GDP-fucose, by the action of epimerases, and this seems to be the only natural source of fucose for glycoproteins (23). However, if exogenous fucose is injected into animals it is first conjugated intracellularly to form fucose-1-phosphate (24) followed by conversion into GDP-fucose (25) and, finally, this nucleotide-sugar functions as a sugar donor in glycoprotein synthesis. Exogenous fucose administered to animals is not converted into other sugars or substances of another nature but is recovered from the urine and feces in its original form. Only 2% of injected fucose is converted into water and carbon dioxide within 10 h of injection. Therefore, the sole fate of [^{3}H]-fucose injected into animals is to be incorporated as such into newly synthesized glycoproteins (26-29).

Material and Methods

In order to evaluate the advantages of [^{3}H]-fucose over the other monosaccharides for the investigation of glycoprotein synthesis, the following experiments were carried out. One of the following sugars was injected intravitreally into young adult male rabbits: L-[^{3}H]-fucose, D-[^{3}H]-galactose, D-[^{3}H]-mannose, N-acetyl-D-[^{3}H]-glucosamine and N-acetyl-D-[^{3}H]-mannosamine. The bilateral intravitreal injections were carried out keeping the rabbits under general anesthesia with sodium thiopental, and each radioactive sugar was dispensed in 20 µl saline. The amount of injected radioactivity was either 20 or 200 µCi/eye of each sugar. The rabbits were killed 40 h after the intravitreal injections, and the vitreous body of each eye was processed for electrophoresis and fluorography, and the surrounding tissues such as retina and ciliary body were processed for radioautography. Each vitreous sample used for electrophoresis and fluorography contained 30 µg of protein. Other samples were used for radiometric measurements and the specific activity of the proteins is reported as counts per minute (cpm) per µg protein (30,31).

In another series of experiments the incorporation of [^{3}H]-fucose into vitreous proteins was compared to that observed for amino acids such as [^{3}H]-proline, [^{3}H]-tyrosine and a solution containing 15 different amino acids. The experimental design was the same as used for the above five sugars (32).

In both series of experiments, the fluorographs of the polyacrylamide gels and the radioautographs of duplicate gels containing identical vitreous samples were compared. For the fluorographic technique (33), the gel is soaked in a solution containing diphenyloxazole, a fluorochrome, and then placed in contact with a film (e.g., Kodak X-OMAT-AR5). For direct radioautography the dried gel is pressed directly against an appropriate film (e.g., Hyperfilm, Amersham, Buckinghamshire, England).

Results and Discussion

No radioactive bands were detected in the lanes containing vitreous samples from rabbits injected intravitreally with either [^{3}H]-Man or [^{3}H]-Gal, even when the dose of each sugar was 200 µCi/eye. A few bands showing very low activity were detected in eyes injected with 200 µCi of N-acetyl-D-[^{3}H]-mannosamine, but no activity was observed at lower doses. Radioactive bands were conspicuous in lanes corresponding to samples of the 200 µCi-[^{3}H]-GlcNAc-injected eyes and their intensities were comparable to,

although lighter than, the vitreous samples of eyes injected with 20 µCi of [^{3}H]-Fuc. In short, after the intravitreal injection of 20 µCi of [^{3}H]-Fuc, 14 radioactive bands were detected in the fluorographs of the polyacrylamide gels bearing samples of the vitreous body. This was never matched by any of the other sugars used in similarly designed experiments (Figure 1).

Labeling of the vitreous proteins was observed after the intravitreal injection of tritiated amino acids. The specific activity of the vitreous glycoproteins labeled after injection of 20 µCi [^{3}H]-Fuc was much higher than that found after the same dose of any of the tritiated amino acids. The specific activity of the [^{3}H]-tyrosine- or [^{3}H]-proline-labeled proteins was about 11 times lower than that of the [^{3}H]-fucose-labeled glycoproteins. Even when 100 µCi of each individual amino acid or the amino acid mixture was used per eye, the specific activity obtained for the [^{3}H]-fucose-labeled glycoproteins following the 20-µCi dose was at least twice as high as that verified for any amino acid (Figure 2).

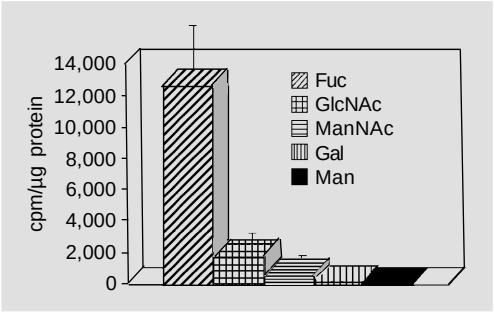

Figure 1 - Specific activity of rabbit vitreous glycoproteins 40 h after intravitreal injection of 200 µCi of one of the following tritiated sugars: fucose (Fuc), N-acetyl-glucosamine (GlcNAc), N-acetyl-mannosamine (ManNAc), galactose (Gal) or mannose (Man). Data are reported as means ± SD for 5 determinations.

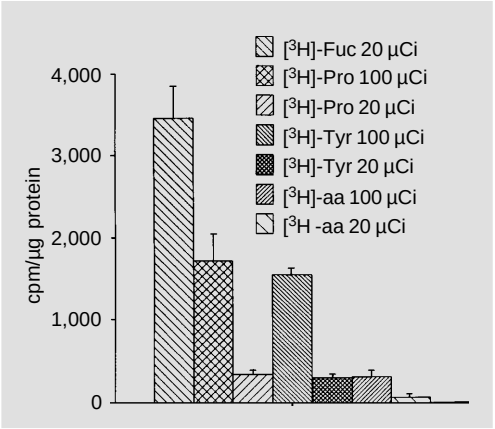

Figure 2 - Specific activity of rabbit vitreous proteins 40 h after intravitreal injection of [^{3}H]-fucose ([^{3}H]-Fuc) (20 µCi), [^{3}H]-proline ([^{3}H]-Pro) (100 or 20 µCi), [^{3}H]-tyrosine ([^{3}H]-Tyr) (100 or 20 µCi) or a [^{3}H]-amino acid mixture ([^{3}H]-aa) (100 or 20 µCi). Data are reported as means ± SD for 5 determinations.

With very few exceptions, the labeled bands detected in the fluorographs of the [^{3}H]-fucose-labeled vitreous samples were also observed for the amino acid experiments. Of course, the labeling was much lighter in the amino acid-injected eyes.

In general, a higher sensitivity was observed for the fluorographs of the gels (33) when compared to the corresponding radioautographs where no "enhancer" was interposed between the film and the gel. However, the profiles of the bands showing very high specific activities were not very well defined in the fluorographs. The same was observed when long exposure times were employed.

There was a good correlation between the specific activity of the vitreous proteins and the silver grain density of several tissues located around the vitreous body, as revealed by radioautography on semi-thin and paraffin sections of the ciliary body, retina and lens.

The very high specific activities obtained with [^{3}H]-fucose-labeled glycoproteins when this precursor is administered to animals *in vivo* (intravenously or intraluminally) or *in vitro* create very favorable conditions for radioautographic (34) studies of these macromolecular compounds, particularly those related to the secretion or biogenesis of glycoproteins. Thus, radioautography helps clarify the sites of synthesis and the intracellular pathway of glycoproteins destined for lysosomes and the plasma membrane (12). For the latter, a study was performed involving about 50 different animal cell types (35). The importance of this study was demonstrated by the numerous citations of this particular paper, as verified by the Institute for Scientific Information (Current Contents, Life Sciences, Vol. 32, No. 44, 1989). The contribution of radioautography to the clarification of the role of the Golgi apparatus in

protein secretion was of crucial importance and the experimental design became less troublesome and more reliable with the availability of radioactive fucose (36-39). There are detailed studies using electron microscopic radioautography to clarify the cellular process of glycoprotein secretion in some particular cell types, such as the thyroid follicular cells (40), ameloblasts (41), odontoblasts (42) and osteoblasts (43), the secretory neurons of the supraoptic and paraventricular nuclei (44), the acinar (45) and the endocrine (46) cells of the pancreas, the lens epithelium (47), the epithelial cells lining the granular ducts of the submandibular glands (48) and the uterine tubes (49) of the mouse, among others. All the above studies were performed using [^{3}H]-fucose for labeling the newly synthesized glycoproteins. Straightforward results can also be obtained using radioautography on semi-thin sections (50) and several studies using this technique have been undertaken to analyze cell secretion. Among these investigations, we should cite one that clearly contributed to tracing the origin of the zona pellucida (51). More recently this same technique – providing high resolution and a minimum of artifacts – has been used in our research on the origin of vitreous body glycoproteins (30,52).

In conclusion, radioactive fucose is the precursor of choice when experiments on glycoprotein synthesis and secretion are designed. This hexose may not be used only in cases when it is known beforehand that the carbohydrate side chains of a particular glycoprotein or a group of glycoproteins does not contain it. The specific activity of the glycoproteins obtained with [^{3}H]-fucose administration is much higher than that observed for other hexoses or any amino acid. This latter point is also true for plasma proteins, as observed in our laboratory several years ago. When administered systemically, radioactive fucose must be injected intravenously and not intraperitoneally in order to obtain a very high specific activity of this precursor both in plasma and intracellularly. Fucose is not converted to other sugars or substances of another nature. Therefore, radioactive fucose is a very reliable and specific precursor for labeling newly synthesized glycoproteins. Due to the higher sensitivity of radioautography over other histochemical methods (PA-Schiff, lectins, etc.) for the identification of carbohydrates (53), the administration of [^{3}H]-fucose followed by radioautography may be used as a tool for the identification of glycoproteins in tissues where these compounds occur only in minute concentrations. Finally, in the present study we point out that important issues of cell biology such as the biogenesis of lysosomal and membrane glycoproteins as well as the cellular processes of protein secretion have been unraveled in part with the use of radioautography in association with the administration of [^{3}H]-fucose.

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