Pathologie Biologie 60 (2012) 66–75

Available online at
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

50th Anniversary – French Society for Connective Tissue Research

Fifty years of structural glycoproteins

Cinquante ans de glycoprotéines de structure

J. Labat-Robert, L. Robert *

Laboratoire de recherche ophtalmologique, hôpital Hôtel-Dieu, université Paris-5, 1, place du Parvis-Notre-Dame, 75181 Paris cedex 04, France

ARTICLE INFO

Article history:
Received 1 April 2011
Accepted 30 October 2011
Available online 9 January 2012

Keywords:
Connective tissue
Glycoproteins
Acute phase
Cornea
Structural glycoproteins
Immunology
Graft rejection
Fibronectin
Cell-matrix interactions

ABSTRACT

During decades preceding and following the last war, a favourite subject of biochemists was to study glycoproteins. One class of these substances, found in connective tissues were characterised as polysaccharides, most of them found to be linked to proteins, designated later as glycosaminoglycans and proteoglycans. Another family of glycoconjugates represented epithelial mucins as found in the gastro-intestinal and respiratory tracts and conduits. A third family of glycoconjugates is represented by circulating glycoproteins isolated from the blood plasma, mostly studied by medical biochemists in relation to pathological conditions comprising those increasing during the inflammatory reaction: acute phase glycoproteins. Their study suggested that they might be derived from connective tissues. Although inflammatory glycoproteins derive mostly from the liver, the possibility of connective tissue origin of glycoproteins remained open. Using cornea, an avascular tissue, we could show that connective tissues also synthesize glycoproteins. We proposed to designate them "structural glycoproteins" (SGP-s) to distinguish them from circulating, blood-born glycoproteins coming from the liver. They play locally "structural" roles in connective tissues where they are synthesized. Soon after fibronectin was identified and shown to mediate cell-matrix interactions. A large family of glycoproteins were then isolated from a variety of sources, cells, tissues others than liver, confirming our original hypothesis. The first experiments on these glycoproteins were published from 1961/1962 giving the opportunity to recapitulate this biochemical adventure 50 years later, together with the celebration of the foundation of the first connective tissue society in Europe, as described in the first article in this issue.

Mots clés:
Tissus conjonctifs
Glycoprotéines
Phase aiguë
Cornée
Glycoprotéines de structure
Immunologie
Rejet de greffes
Fibronectine
Interactions cellules-matrice

RÉSUMÉ

Au cours des décennies avant et après la dernière guerre, l'un des sujets favoris des biochimistes a été l'étude des glycoconjugés. Une des familles de ces substances, des polysaccharides liés le plus souvent à des protéines est devenue : les glycosaminoglycannes et protéoglycannes des tissus conjonctifs. Une autre famille correspond aux mucines épithéliales. Une troisième famille est représentée par les glycoprotéines du sang circulant. Le processus pathologique le plus étudié dans ce contexte a été le phénomène inflammatoire. L'étude des glycoprotéines sériques inflammatoires a suggéré que cette augmentation de leur concentration sérique peut avoir son origine dans les tissus conjonctifs, les plus souvent impliqués dans ces processus pathologiques. Bien qu'il a été montré ultérieurement que la plupart des glycoprotéines inflammatoires proviennent du foie, une origine du conjonctif de certaines glycoprotéines est restée une hypothèse plausible. Utilisant les cornées avasculaires et des traceurs radioactifs, nous avons pu montrer que les cellules des tissus conjonctifs produisent des glycoprotéines désignées : glycoprotéines de structure (GPS) pour les distinguer des glycoprotéines circulatoires d'origine hépatique. Ces glycoprotéines tissulaires jouent des rôles localement, au niveau de leur site de synthèse, dans la formation des tissus conjonctifs. Avec la découverte de la fibronectine cette thèse a été largement confortée, suivie par l'identification d'autres glycoprotéines d'origine tissulaire, locale. Nos premières publications sur ce sujet datent des années 1961/1962, c'est la raison de présenter cette revue sur leur origine dans ce numéro du journal consacré au cinquantenaire de la fondation de la Société française des tissus conjonctifs.

* Corresponding author.
E-mail address: lrobert@wanadoo.fr (L. Robert).

0369-8114/$ – see front matter © 2011 Published by Elsevier Masson SAS.
doi:10.1016/j.patbio.2011.11.010

1. Introduction

During the early years of biochemical research the study of sugars played for some time a central role. The constance of blood sugar, demonstrated during the second half of the 19th century by Claude Bernard [1] and confirmed by several other investigators, was followed by the demonstration of its "polymeric" storage (as glycogen) in the liver and some other tissues as the muscles. Glucose was also the most intensely studied metabolite during the early years of investigations of what became designated as the intermediary metabolism. This early history of the progressive discovery of the details of glucose metabolism is recounted in detail by Bierry, professor of physiology at the Science Faculty of Marseille and Rathery, professor of medicine at the Paris Medical Faculty in 1935 [2]. One can be surprised by the complexity of these early studies which yielded finally the description of glycolysis and later the oxidative metabolism of carbohydrates. These same authors, Bierry and Rathery, devoted more than the half of their treatise to the protein-bound form of carbohydrates, designated as the "sucre protidique" or "protein-bound sugar" [2]. They proposed a method for its determination and explored a large number of pathological situations such as cancer, diabetes, infectious diseases, nephritis and more. They made several important observations as for instance the independent variation of free sugar and protein-bound sugar in pathological conditions as well as after the administration of hormones or drugs as adrenaline and others. Their experiments were carried out before the elucidation of the protein composition of blood plasma which started only after the war with the discovery of electrophoresis and even more, of immune-electrophoresis at the Pasteur Institute by the Grabar school [3]. This is certainly the main reason for the statements concerning euglobulins and pseudoglobulins and their sugar content [2]. Nevertheless these authors made a number of important discoveries. They showed, among others, that protein bound sugar (glycoproteins) is present in the body fluids of all animal species investigated from molluscs to humans. The (total) destruction of the liver by formol injections decreased drastically the level of protein- bound sugar in the blood. Some hormones as for instance an extract of anterior hypophysis increased their blood concentration without affecting free blood sugar [2]. They also carried out experiments (and cited similar experiments by other authors) to see if free sugar can combine in solution with proteins. They found that they could, but this combination of glucose with "albumin" could be easily split with dilute acid treatment. They concluded that free sugar, although capable to combine with proteins, is not at the origin of protein-bound sugar as found in the blood. This is probably the first indication of glycosylamine formation between reducing sugars as glucose and free amino-groups on proteins as confirmed later, following the discovery by Maillard of the reaction named after him [4, for a review]. These early studies and their successful medical application as well as their central role in the elucidation of sugar metabolism are the obvious reasons of the popularity of studies on glycoconjugates, as they were later designated in biochemistry in general and in medical biochemistry in particular.

2. Inflammatory glycoproteins

With the advent of electrophoretic separation of proteins and of immuno-electrophoresis, it became evident that "protein-bound sugar" consists of a large number of individual glycoproteins. They were successively isolated and characterised as initiated by the ambitious program of blood fractionation by Cohn and Edsall during the last world war in Boston [5]. It became progressively easier and more precise to determine glycoproteins as compared to the determination of protein-bound sugar as done previously [2]. These studies resulted in the characterization of the glycoproteins which most strongly increase in pathological conditions. Among them "orosomucoid" as designated by Richard Winzler [6] and studied in great detail by Karl Schmid [7], played a crucial role. This α_1-acid glycoprotein was shown to increase in a number of pathological conditions. Being rich in carbohydrates, it represented a relatively important part of "protein-bound sugars" as well as of the serum fractions soluble in sulfosalicylic acid (called also seromucoid) strongly enriched in sugar-rich glycoproteins. Orosomucoid was one of its main constituents. These determinations, carried out routinely in hospital-based biological laboratories, became even easier after the discovery of the ELISA-methodology, using specific antibodies.

For routine determinations of this trichloro- perchloro- or sulfosalicylico-soluble "seromucoid" fraction we introduced a new method based on their polarographic determination [8–10]. In order to characterize the glycoproteins giving the polarographic waves recorded in these acid-soluble extracts, combined immune-electrophoretic and polarographic methods were used. Immunee-lectrophoresis confirmed the presence of orosomucoid, α_1-acid glycoprotein as the main component, confirming the relative specificity of this method [11]. We also could demonstrate a polarographic reaction attributable to sialic acid [12]. These comparative studies confirmed orosomucoid (α_1-acid glycoprotein) being the principal constituent of the perchloric acid soluble serum fraction and also of the trichloracetic acid soluble fraction. The sialic acid content did not critically influence the height (in μA) of the polarographic wave, only the elctrophoretic mobility. For the characteristics of the polarographic wave the nature, composition and structure of the glycan and protein portions were more important [12]. Later the use of specific antibodies simplified this procedure.

3. Haptoglobin

A special case emerged during these investigations of the pathological significance of blood-born glycoproteins with the discovery of haptoglobin by M.F. Jayle. He could show that a specific serum glycoprotein, migrating on electrophoresis as an α_2-glycoprotein, combines specifically with free hemoglobin. The hemoglobin-haptoglobin complex (HbHp) exhibited a strong, specific hydroperoxidase activity. This enabled its specific determination in small blood-serum samples without its previous isolation [13]. Haptoglobin behaved also as an inflammatory glycoprotein and could be determined in the sera of a large number of patients. These determinations, routinely carried out in Jayle's Department of Biochemistry at the Paris Medical Faculty, yielded a number of important informations on the role of the inflammatory process in diseases. Inflammation was characterized since the classical Greek school of Hippocrates as the redness and swelling of tissues with pain and dysfunctions, as learned by every medical student. Professor Jayle, who lost his eyes as a young assistant professor when preparing ethylperoxide, the substrate of the HbHp – peroxidase test, elaborated a hypothesis during the 1950s, attributing a local, tissue-origin of inflammatory glycoproteins. To prove this hypothesis he proposed to use animal and in vitro experiments. Several interesting experiments were carried out during this period as for instance the use of the carrageenine-induced granuloma in rats or guinea-pigs. The subcutaneous injection of this seaweed polysaccharide was shown to produce a rapid connective tissue proliferation followed by its resorption [14]. We could show that this process was accompanied by a strong increase of the circulating inflammatory glycoproteins and especially haptoglobin [15]. We also could study the tissue proteases involved in the resorption of the carrageenin-induced neosynthesis of connective tissues [16]. This model proved ideal