An abundant ubiquitous glycoprotein (GP_{100}) in nucleated mammalian cells

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Two-dimensional gel electrophoresis with the ^{125}I-Con A overlay and affinity purification with Con A-agarose revealed the presence of an abundant ubiquitous 100-kDa glycoprotein (GP_{100}) in nucleated mammalin cells. The amount in cultured human and murine cells varies from 3 to 20 $\times$ 10^6 molecules per cell making GP_{100} the most abundant glycoprotein in nucleated cells. Peptide mapping shows that it is different from erythrocyte Band III protein. Several properties of GP_{100} suggest that it could play a structural role in nucleated cell membranes.

Con A receptor glycoprotein *Abundant glycoprotein* *GP_{100}* *Common glycoprotein* *Nucleated cell glycoprotein* *Membrane structure*

1. INTRODUCTION

Anucleate cells such as erythrocytes and platelets contain abundant Con A acceptor glycoproteins, e.g., erythrocyte Band III protein [1], platelet GPI-IV [2]. Band III protein is a multifunctional protein which acts as an anion channel [3], is the attachment site for the cytoskeleton [4] which controls the fluidity and shape of the cell membrane and is the attachment site for other proteins to the cell membrane [5]. Thus these abundant glycoproteins appear to play a significant structural role in anucleate cell membranes.

In contrast, analogous abundant glycoproteins have not been identified in nucleated cells. Most of the well characterized membrane glycoproteins of nucleated cells, e.g., surface antigens and receptors, exist at levels around 1–5 $\times$ 10^5 molecules/cell [6]. Furthermore, many of these glycoproteins are not expressed by all cells and are therefore unlikely to perform general structural roles in nucleated cells.

Abbreviations: Con A, concanavalin A; PMSF, phenylmethylsulfonyl fluoride; TLCK, *p*-tosyl lysyl chloroketone

Here it is shown that nucleated cells from several mammalian species do express a major glycoprotein which is one of the most abundant cellular proteins. The protein resembles erythrocyte Band III protein in several respects, but the available evidence indicates that they are different proteins. A glycoprotein with comparable abundance and ubiquity in nucleated mammalian cells has not been described previously.

2. MATERIALS AND METHODS

Two-dimensional fingerprinting and ^{125}I-Con A overlay were carried out as in [7]. Samples of cells or tissues were lysed in 2% Nonidet P40 as in [7].

Con A-agarose was prepared from purified Con A (Pharmacia) and CNBr-activated Sepharose as prescribed by the manufacturers. Glycoproteins were eluted from Con A-agarose with 10% α-methyl mannoside in phosphate-buffered saline (PBS).

SDS-gel electrophoresis was carried out as described by Laemmli [8]. Peptide mapping was carried out according to the method of Cleveland et al. [9]. Samples of purified glycoproteins or murine red cell membranes (for Band III protein)

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were run on an SDS gel, the appropriate protein band excised and re-run on an SDS gel after papain digestion. The peptide patterns were developed with the silver stain [10].

3. RESULTS

The existence of an abundant glycoprotein in nucleated cells was first evident from 2D fingerprinting studies of cultured tumour cells (fig.1). A major 100-kDa protein with an isoelectric point of pH 5.3–5.4 (i.e., between α- and β-tubulin) was also found to stain with the ^{125}I-Con A stain for glycoproteins. In this respect the glycoprotein is virtually unique since none of the other glycoproteins detected by the lectin reagent is of sufficient abundance to also stain with the Coomassie blue protein stain.

The existence of the abundant 100-kDa glycoprotein (GP_{100}) was also demonstrated by Con A affinity purification from lysates of nucleated cells (fig.2). The eluates from the Con A-agarose show a 100-kDa protein which is clearly the most abundant component to stain with the protein stain. Quantitative densitometry of such gels shows that the 100-kDa glycoprotein can

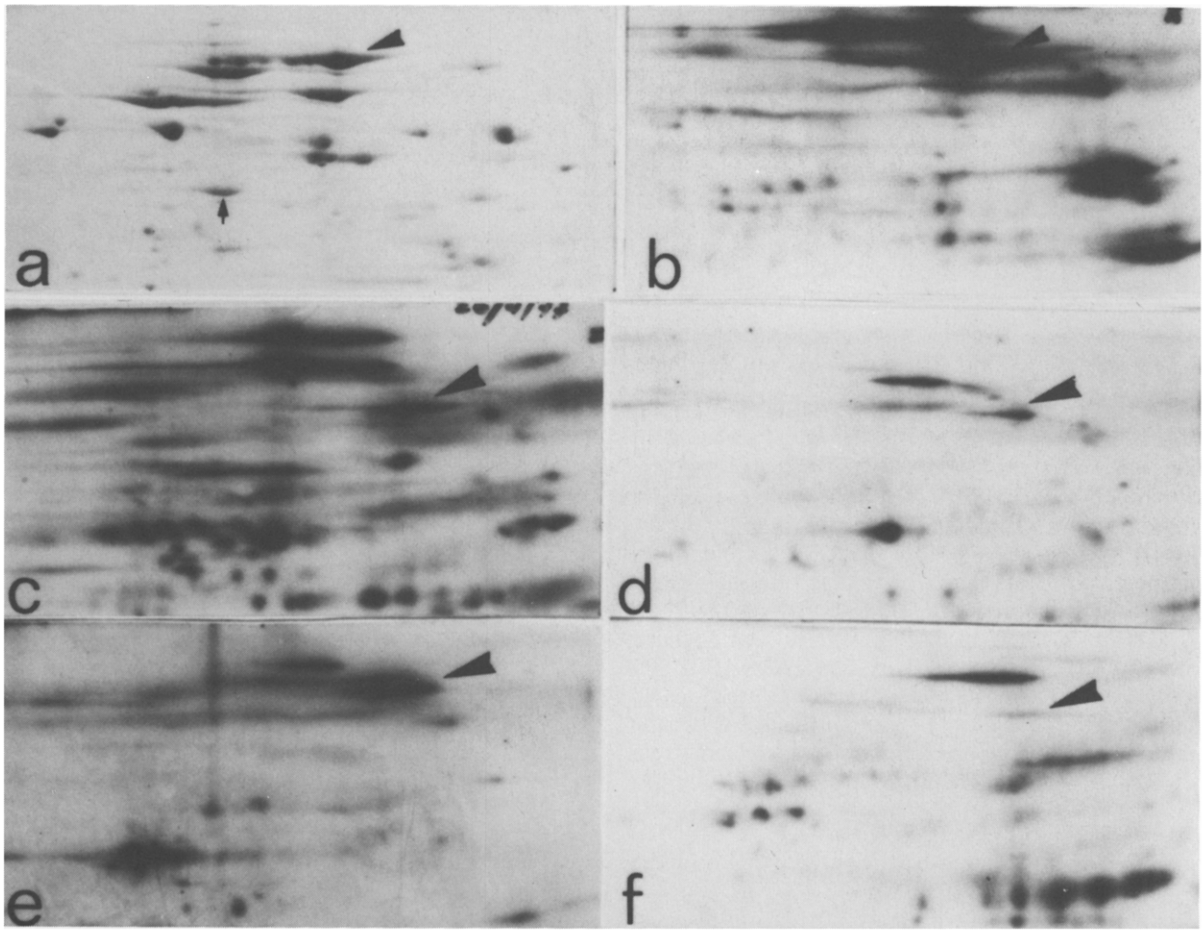

Fig.1. Identification of an abundant 100-kDa glycoprotein GP_{100} in nucleated cells by 2D gel electrophoresis and ^{125}I-Con A overlay. Cell or tissue lysates were prepared and analysed as described [7]. Each gel was stained for protein and glycoprotein with Coomassie blue and ^{125}I-Con A, respectively. The arrowhead points to the position of a 100-kDa protein (pI = 5.4) which is stained by both protein and glycoprotein stains. In all cases the origin (basic end) is at the top left of each panel. (a) Protein stain. (b–f) Glycoprotein stain. (a + b) Murine X63 myeloma. (c) Murine C3H L cells. (d) Murine lymphocytes. (e) Rabbit lymphocytes. (f) Human brain.

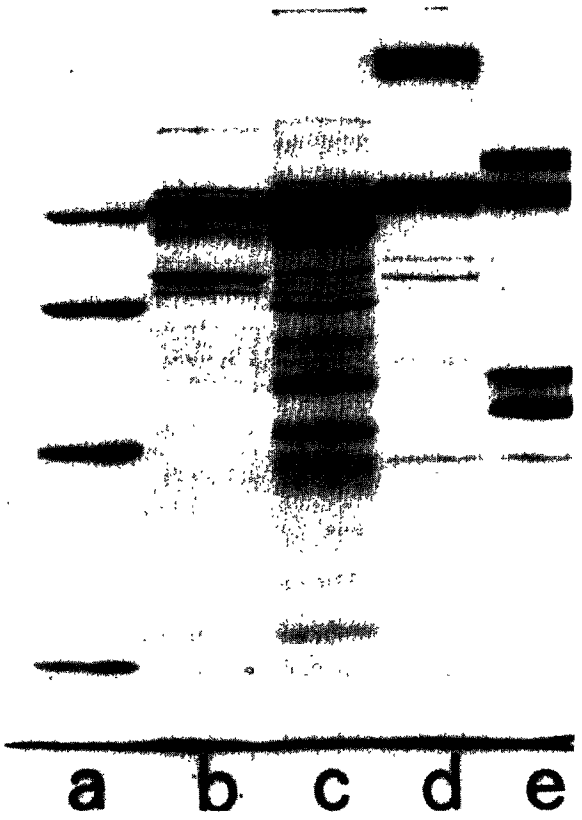

Fig.2. Identification of GP_{100} by affinity purification with Con A agarose. Cell lysates were prepared in phosphate-buffered saline/2% NP40 with protease inhibitors (1 mM PMSF, 2 mM benzamidine, 5 mM *o*-phenanthroline, 1 mM TLCK) mixed with Con A agarose and eluted with 10% α-methyl mannoside in phosphate-buffered saline. The eluates were analysed by SDS-gel electrophoresis [8]. (a) Molecular mass markers (95 kDa, 65 kDa, 45 kDa, 30 kDa). (b) Con A eluate from murine macrophage cell line (A2/C12.2). (c) Con A eluate from HeLa cell line. (d) Unfractionated murine erythrocyte membranes showing Band III, the major 100-kDa glycoprotein. (e) Con A eluate from human platelets.

account for up to 80% of the total protein in the eluate from the Con A-agarose.

The absolute levels of GP_{100} in four cultured cell lines were determined by quantitative extraction and elution from Con A-agarose followed by SDS-gel electrophoresis and quantitative densitometry. The yields were calculated in terms of the number of molecules per cell and were 3×10^6 for the P815 mastocytoma cell, 2.7×10^6 for the HeLa cell, 9.4×10^6 for the X63 myeloma cell and 1.7×10^7 for the MOPC 315 myeloma cell.

When cells are subjected to hypotonic lysis followed by mechanical disruption to separate the cytosol, membranes and nuclei, >90% of the GP_{100} is found in the membrane fraction showing that it is associated with cell membranes. However, it has not been established yet whether it is associated with all cell membranes or with a particular membrane compartment in nucleated cells.

The second interesting property of GP_{100} is its expression in a wide variety of nucleated cells. Table 1 summarises the results of an extensive survey using the 2D gel/Con A overlay procedure on a variety of cells and tissues. In each case the

Table 1

Expression of GP_{100}-like glycoprotein in various cells and tissues

Sample	GP_{100} expression
Human lymphocytes	+
Murine lymphocytes	+
Rat lymphocytes	+
Rabbit lymphocytes	+
Murine thymocytes	+
Human platelets	–
Human erythrocytes	–
Murine erythrocytes	–
Chicken erythrocytes	+
Human brain	+
Rat brain	+
Xenopus oocytes	+
Human fibroblastoid cultured cells	+
Murine fibroblastoid cultured cells	+
Human lymphoblastoid cultured cells	+
Murine lymphoblastoid cultured cells	+
Rat lymphoblastoid cultured cells	+
Murine macrophage cultured cells	+
Human epithelial cultured cells	+
Human neuroblastoid cultured cells	+
Drosophila cultured cells (KCO)	–
Drosophila sperm cells	–
Nematode (*C. elegans*) extracts	–

Nonidet P40 lysates of all samples were subjected to 2D-gel electrophoresis and ^{125}I-Con A overlay. GP_{100} was identified by its position on the 2D gel (apparent molecular mass 100 kDa, pI 5.3–5.4) staining with Coomassie blue and ^{125}I-Con A

glycoprotein was identified by four criteria: apparent molecular mass, 100 kDa; p*I*, 5.3–5.4; staining by the Coomassie blue reagent; and staining by ^{125}I-Con A overlay. It is apparent that the glycoprotein exists in all mammalian nucleated cells examined so far. These include both normal and transformed cells, cells grown in vitro and in vivo as well as normal tissue samples, i.e., human and rat brain. A similar glycoprotein has also been detected in Xenopus oocytes and chicken red blood cells but so far a glycoprotein satisfying all four criteria for GP_{100} has not been detected in invertebrate cells.

GP_{100} resembles Band III protein from erythrocytes in size, abundance and Con A binding (fig.2). To test whether they had any similarity in their sequences, the two proteins were compared by peptide fingerprinting (fig.3). The results show that whereas GP_{100} from murine or human cells are virtually indistinguishable, there is no obvious similarity with the pattern of fragments obtained from Band III protein. Thus, apart from their superficial similarity, GP_{100} and Band III protein appear to be distinct proteins. The considerable similarity between the patterns obtained from murine and human GP_{100} indicate that it is relatively conserved in its amino acid sequence in different species. This is also suggested by the fact that the glycoprotein occupies the same position on the 2D maps of cells from different species.

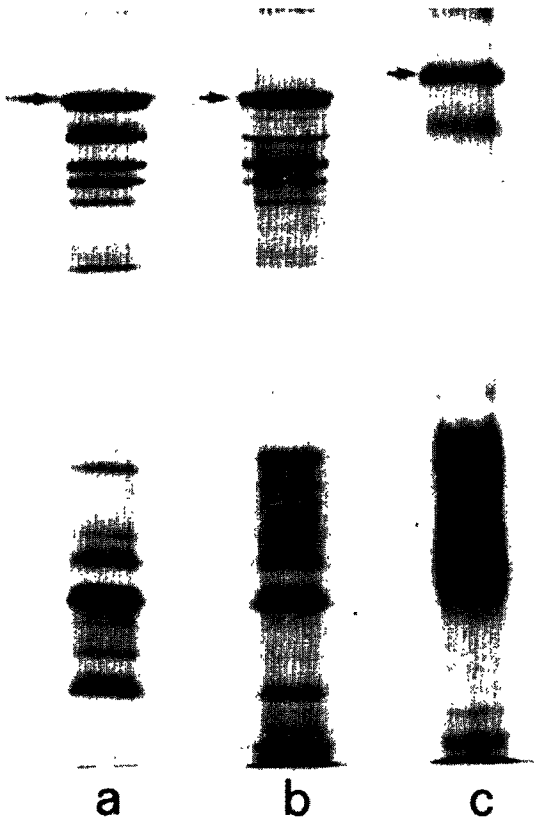

Fig.3. Peptide fingerprinting of GP_{100} and Band III protein. Samples were analysed by the method of Cleveland et al. [9] as described in section 2. In each case the position of the uncleaved protein is shown. (a) GP_{100} band from murine macrophage cell line (A2/C12.2). (b) GP_{100} band from human HeLa cell line. (c) Murine Band III protein.

4. DISCUSSION

The purpose of this report is to draw attention to the fact that nucleated cells from a variety of mammalian species express an abundant 100-kDa glycoprotein. Although the glycoprotein resembles erythrocyte Band III protein in several respects, the available evidence indicates that they are different proteins.

Although extensive studies have been carried out on glycoproteins from nucleated mammalian cells, this is the first report of an abundant, common glycoprotein in such cells. It was previously shown that murine L cells contain an abundant ($>1 \times 10^6$ molecules/cell) 100-kDa Con A acceptor glycoprotein [11] which shows many similarities with GP_{100}. However, the amino acid analysis of the L-cell glycoprotein is significantly different from that of GP_{100} (unpublished) and its presence in other cells was not investigated. Hughes and August [14] identified a major surface glycoprotein in murine fibroblasts and other cells using monoclonal antibodies. However, the glycoprotein is clearly larger than GP_{100}, and is much more heterogeneous on 2D and 1D gels. Furthermore, the 110-kDa glycoprotein could not be detected in murine thymus whereas GP_{100} is clearly present in murine thymocytes (see table 1). Thus the available evidence suggests that GP_{100} has not been described previously. One contributing factor to this could be the exceptional sensitivity of GP_{100} to proteolysis, especially by Ca^{2+}-activated proteases (unpublished). Thus the mixture of protease inhibitors described in the legend to fig.2 was found

to be essential for obtaining adequate yields of the glycoprotein during affinity purification.

The function of GP_{100} is not known. However, many of its known properties suggest that it might play a structural role in cells. Thus most of the other abundant proteins in cells, e.g., actin [12] and tubulin [13], are involved in the formation and maintenance of some type of intracellular structure. It is generally assumed that the high degree of sequence conservation they manifest reflects their need to interact with other proteins when they perform a scaffolding function in cells. By analogy, it is expected that one of the roles of GP_{100} will be as a structural protein. It is also noteworthy that GP_{100} is most abundant in secreting cells such as plasmacytomas (see fig.1) and that preliminary immunofluorescence studies show that it is mostly in intracellular membrane vesicles (unpublished). Thus, GP_{100} might be involved in the assembly and function of intracellular vesicles during secretion and other related processes.

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