POSITION OF GLYCOPROTEIN POLYPEPTIDE CHAIN IN THE HUMAN ERYTHROCYTE MEMBRANE

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1. Introduction

A study of the vectorial arrangement of the protein of human erythrocyte membrane showed that the 90,000 molecular weight class of protein was exposed on the ouside of the red cell membrane [1, 2]. This observation is based on the ability of the enzyme, lactoperoxidase, to catalyze the iodination of protein of this molecular weight class on the intact human erythrocyte. The analysis of the membrane protein separated on 5% acrylamide gels employed in these studies showed a glycoprotein present in the region of 90,000 molecular weight proteins. Recent studies have shown that glycoproteins have anomolous behavior on SDS disc gel electrophoresis systems [3, 4]. Cross-linking of the acrylamide gel in this system does not, however, affect the molecular weight estimation of protein.

In order to extend our observations on the proteins that are iodinated by lactoperoxidase on the intact membrane, we have re-examined the membrane protein employing SDS electrophoresis on polyacrylamide gels with varying cross linkage. These studies have confirmed that a 90,000 molecular weight protein is exposed on the human erythrocyte membrane. In addition, a glycoprotein with an apparent molecular weight of 62,000 as determined by 10% disc gel electrophoresis is labeled and also occupies an exposed position on the membrane.

2. Materials and methods

All chemicals employed in these studies were reagent grade. Carrier free ^{125}I was purchased from Schwarz Chemicals. Lactoperoxidase was isolated by the method of Morrison and Hultquist [5]. Human erythrocyte membranes were prepared by the method of Dodge et al. [6] as modified by Phillips and Morrison [1]. Washed, intact erytrocytes were catalytically iodinated with ^{125}I by lactoperoxidase as previously described [1]. The iodinated erythrocyte membrane fraction was dialyzed against 5 mM mercaptoethanol, 5 mM EDTA (pH 7.4) for 18 hr, solubilized in 2.5% SDS and electrophoresed on 10% polyacrylamide gels containing 0.1% SDS [1]. Gels were stained for protein with Coomassie blue and for carbohydrate with fuschin sulfate. The distribution of radioactivity in the gel was determined from the gel slices.

3. Results

The stroma isolated from iodinated intact erythrocytes were solubilized in SDS and fractionated by electrophoresis on 10% polyacrylamide gels. Gel A in fig. 1 has been stained for carbohydrate and four bands are clearly visible, corresponding to molecular weights of 62,000, 34,000, 29,000 and 14,000, respectively. The 14,000 molecular weight material, which has been reported by various investigators to be lipid [7] or glycolipid [8], stains abnormally and may not be a glycoprotein.

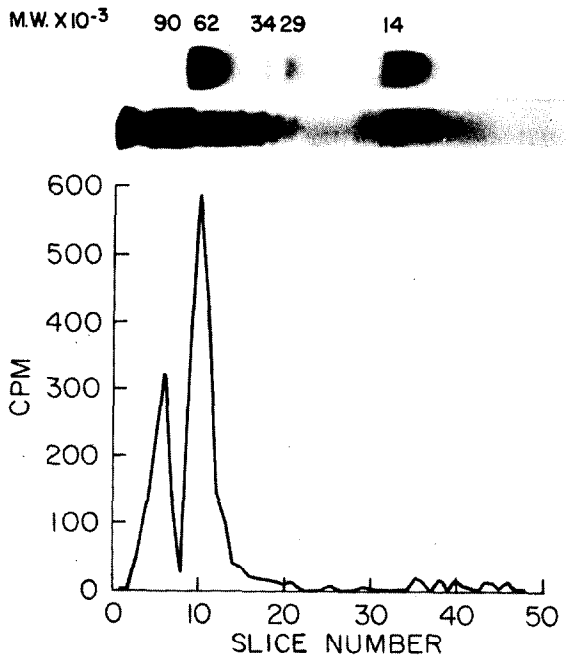

Fig. 1. Electrophoretic separation of membrane proteins from iodinated intact erythrocytes. Membranes from iodinated cells were solubilized in sodium dodecyl sulfate (SDS) and electrophoresed in 10% polyacrylamide gels as described in Methods. The gel at the top was stained for carbohydrate with fuschin sulfate and the one beneath for protein with Coomassie blue. The graph shows the radioactive iodine distributions in the gel. Electrophoresis was from left to right.

Gel B in the figure has been stained for protein with Coomassie blue. The molecular weight distribution of these membrane proteins is similar to that observed on 5% gels. Molecular weight classes of proteins from 200,000 to 20,000 are present as previously reported [1, 4, 7, 8]. The graph in this figure shows the radioactive iodide distribution in the gel. Two protein molecular weight classes, 90,000 and 62,000, contain the radioactive iodide label.

4. Discussion

The results clearly show that, in contrast to our previous observation using 5% gels, two membrane components are separated on 10% gels which contain the iodine label when intact cells are iodinated using lactoperoxidase. These components have apparent molecular weights of 90,000 and 62,000. The labeled component with an apparent molecular weight of 90,000 appears to be protein since no carbohydrate can be detected with the fuschin sulfate stain in this region of the gel. The 62,000 molecular weight component, however, appears to be a glycoprotein since it moves with an apparent molecular weight of 90,000 on a 5% gel [1] and 62,000 on a 10% gel and in both cases stains for carbohydrate.

The two iodinated components must be derived from the exposed surface of the red cell membrane. Previous data have shown that lactoperoxidase must complex the protein which is iodinated [1, 2]. Hence, all membrane proteins which are iodinated must be exposed on the membrane surface and available to complex lactoperoxidase.

The glycoprotein which is iodinated is the major glycoprotein in the erythrocyte membrane and appears to be the same moelcule which has been studied by a number of investigators [3, 4, 9–12]. Various molecular weights have been reported for this material ranging from 31,000 [11, 12] to 160,000 [10]. The higher molecular weights have been determined by SDS acrylamide disc gel electrophoresis while the lower molecular weight was determined from other physical data [11, 12]. It is clear that examination of size of glycoproteins by SDS electrophoresis gives abnormally high molecular weights. For these reasons the size of the minor glycoproteins shown in the figure with apparent molecular weights of 34,000 and 29,000 as determined by SDS electrophoresis on the 10% gels are probably high. These glycoproteins do not appear to contain any radioactive iodine. The protein moiety of these glycoproteins are not available to lactoperoxidase iodination either because they are buried in the membrane structure or prevented from interacting by the carbohydrate moiety.

The carbohydrate residues in the major glycoprotein have been shown to contain some of the antigenic properties of the erythrocyte [9] and an exterior location of the carbohydrate containing part of this molecule is anticipated. However, since the carbohydrates can be removed by proteolytic hydrolysis [8, 9, 13, 14] and are also substrate for lactoperoxidase, a reasonable portion of the polypeptide chain must be exposed on the membrane surface in addition to the carbohydrate residues.

Labeling intact cells with diazonium salt of sulfanylic acid and subsequent membrane characterization has revealed that the label was predominantly in one membrane component which has a molecular weight of 140,000 [15]. This component has recently been identified as a glycoprotein [16] and appears to be the same molecule which is iodinated as suggested by Steck [8]. The difference in the molecular weight determinations may be due to anomolous electrophoresis of the glycoprotein. Although the sulfanylic acid labeled additional membrane components [15], it is interesting that the glycoprotein is the component that is predominantly labeled. Bretscher has recently employed another organic reagent to label intact erythrocytes and has also shown that the glycoprotein is labeled [4]. An additional protein was labeled in these studies which has a reported molecular weight of 105,000. The difference in the molecular weights makes it appear that there is no relationship between the iodinated protein reported in the present communication and the 105,000 molecular weight protein in that study.

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