The Rhesus (D) polypeptide is linked to the human erythrocyte cytoskeleton

K. Ridgwell, M.J.A. Tanner and D.J. Anstee+

Department of Biochemistry, University of Bristol, Bristol BS8 1TD and +South West Regional Blood Transfusion Centre, Southmead, Bristol BS10 5ND, England

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Cytoskeleton preparations derived from lactoperoxidase-radioiodinated human erythrocytes were found to be enriched in a labelled component with the same apparent molecular mass as the Rhesus (D) (Rh(D)) antigen polypeptide. Immune precipitation from the cytoskeleton preparations confirmed that this component is the Rh(D) polypeptide. The results suggest that the Rh(D) polypeptide may be linked to the erythrocyte skeletal matrix. The possibility that the Rh(D) antigen is involved in maintaining the shape and viability of the erythrocyte is discussed.

Antigen *Cytoskeleton* *Erythrocyte* *Membrane protein* *Rhesus polypeptide*

1. INTRODUCTION

Recent studies have identified the polypeptide carrying the human erythrocyte Rh(D) antigen as a 28.5–32-kDa component. This polypeptide can be labelled by lactoperoxidase-catalysed radioiodination of intact erythrocytes, and can be specifically immune precipitated by human anti-Rh(D) serum [1,2]. Further studies have shown that this integral membrane polypeptide is apparently not glycosylated [3]. The functional role of the Rh(D) polypeptide is unknown. Here, we suggest that this component may be a site of linkage of the erythrocyte cytoskeletal complex with the membrane.

2. MATERIALS AND METHODS

Human anti-D serum was kindly provided by Dr S. Moore (South East Regional Blood Transfusion Service, The Royal Infirmary, Edinburgh, Scotland). -D- erythrocytes were provided by Mrs M. Leak, South London Blood Transfusion Centre, Tooting, London, and cDE control erythrocytes drawn at a similar time were available from the South West Regional Blood Transfusion Service, Bristol. Rh_{null} erythrocytes (donor S.S.) were kindly provided by P.D. Issitt, South Florida Blood Service, Miami, USA.

Lactoperoxidase-catalysed radioiodination of intact erythrocytes using $Na^{125}I$ (Amersham International; spec. act. 13.4 Ci/μg) and the preparation of membranes from radiolabelled erythrocytes by lysis in 5 mM sodium phosphate (pH 8.0) were carried out as in [1]. Cytoskeletons were prepared by Triton X-100 extraction of membranes from radiolabelled erythrocytes as in [4]. The results in fig.1 were obtained from cytoskeletons extracted with 1% Triton X-100 in 5 mM sodium phosphate (pH 8.0) containing 1 mM EDTA (pH 8.0), 1 mM dithiothreitol and 0.2 mM phenylmethylsulphonyl fluoride. Where specified 10 mM Tris–Cl (pH 7.0) or 150 mM NaCl was substituted for sodium phosphate in the extraction buffer.

Phospholipids were extracted as in [13] and lipid phosphate determined as in [14]. Immunoprecipitation of radiolabelled components from Triton X-100-solubilized erythrocyte ghosts was performed as in [1]. Immunoprecipitation of radiolabelled components from cytoskeletons prepared from 1 ml membranes was performed similarly except that the initial binding of the an-

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tibody was to the cytoskeleton preparation rather than to intact erythrocytes.

SDS-polyacrylamide gel electrophoresis of samples was carried out as in [5]. After electrophoresis, gels were fixed in methanol/acetic acid/water (5:1:5, by vol.) for 30 min at 55°C, dried and exposed to Kodak XAR-5 film.

3. RESULTS AND DISCUSSION

The red cell cytoskeleton is operationally defined as the residues remaining after non-ionic detergent extraction of human erythrocyte ghosts [6]. Cytoskeletons prepared from membranes derived from radioiodinated intact human erythrocytes of phenotype cDE and -D- were found to be enriched in a radioiodinated band of the same molecular mass as the Rh(D) polypeptide (fig.1). This band contained much more radioactivity in the membranes and cytoskeletons from D- cells than from cDE cells in accord with the significantly greater abundance of Rh(D) antigen sites in -D- cells over cDE cells (see below). Similar experiments with Rh_{null} erythrocytes (which lack all the antigens of the Rhesus blood group system) did not show the presence of this band in the cytoskeletons (not shown). The identification of this labelled component as the Rh(D) polypeptide was confirmed by immunoprecipitation from the cytoskeletal preparation using human anti-Rh(D) serum (fig.1). The labelled band was immunoprecipitated from cytoskeletons from both cDE and -D- erythrocytes and had the same apparent molecular mass as that precipitated from in-

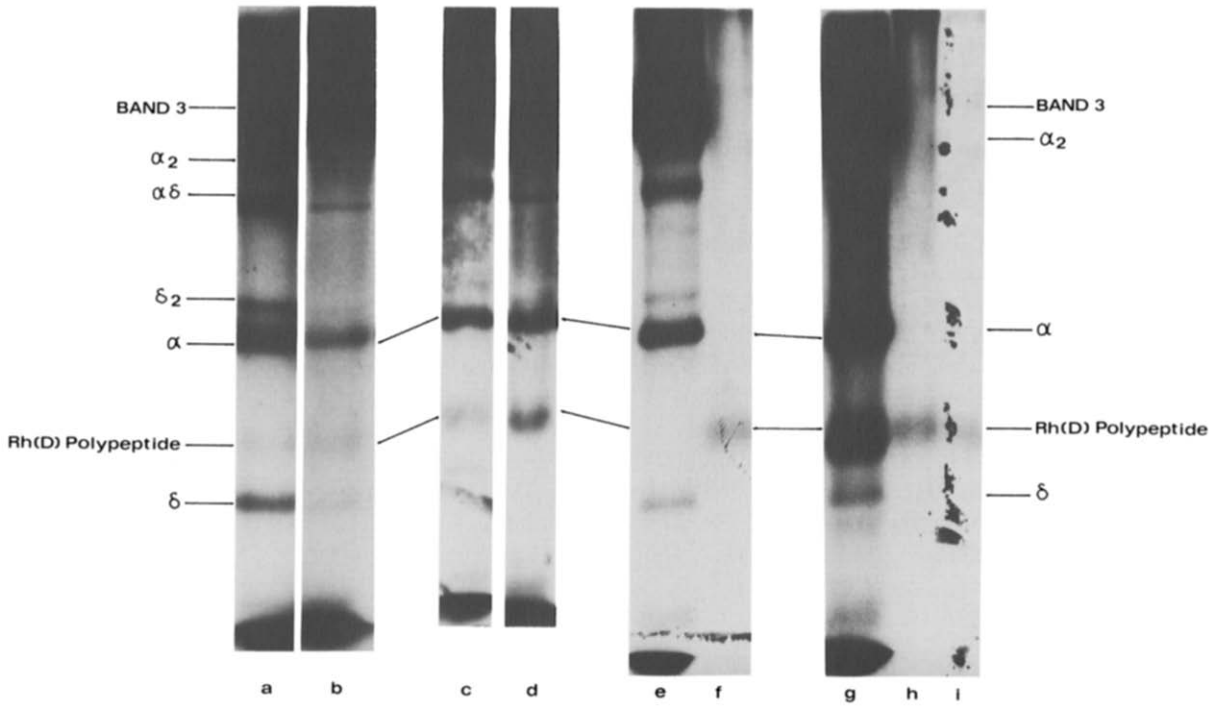

Fig.1. Labelling of intact erythrocytes with ^{125}I, preparation of cytoskeletons from ^{125}I-labelled erythrocyte membranes, and immunoprecipitation of labelled membrane components. (a–d) Autoradiographs of: (a) solubilized membranes from ^{125}I-labelled cDE erythrocytes; (b) cytoskeletons prepared from ^{125}I-labelled cDE erythrocytes; (c) solubilized membranes from ^{125}I-labelled -D- erythrocytes; (d) cytoskeletons prepared from ^{125}I-labelled -D- erythrocytes. Exposure period: (a,b) 5 weeks; (c,d) 2 weeks. (e–i) Autoradiographs of (e) solubilized membranes from ^{125}I-labelled cDE erythrocytes; (f) components from ^{125}I-labelled control erythrocytes precipitated by human anti-Rh(D) serum; (g) solubilized membranes from ^{125}I-labelled -D- erythrocytes; (h) components from ^{125}I-labelled -D- erythrocytes precipitated by human anti-Rh(D) serum; (i) components from cytoskeletons prepared from ^{125}I-labelled -D- erythrocytes precipitated by human anti-Rh(D) serum.

tact cDE and -D- erythrocyte membranes by anti-Rh(D) serum (fig.1).

The results shown in fig.1 were derived from cytoskeletons prepared using extraction of erythrocyte membranes with 1% Triton X-100 in 5 mM sodium phosphate (pH 8.0). The Rh(D) polypeptide was retained in the cytoskeletal preparation even when the Triton X-100 concentration was raised to 5% but was not apparent in cytoskeletons prepared using 10% Triton X-100 (not shown). It was important to show that the Rh(D) polypeptide was not present in the cytoskeletal preparations simply because of hydrophobic association with residual lipid rather than with the cytoskeletal proteins. Since phospholipid analysis could not be satisfactorily carried out on cytoskeletons prepared using extraction in phosphate buffers, similar experiments were carried out with cytoskeletons prepared with 10 mM Tris–Cl (pH 7.0) or 150 mM NaCl. Results were obtained which were similar to those in fig.1 and showed that the Rh(D) polypeptide was retained in cytoskeletons prepared using either 10 mM Tris–Cl or 150 mM NaCl at 1 and 5% Triton X-100, but was not apparent when the Triton X-100 concentration was raised to 10%. The results of phospholipid analysis of these cytoskeleton preparations are shown in table 1. Removal of up to 90% of the ghost phospholipid did not affect the retention of the Rh(D) polypeptide in the cytoskeletons. These results are similar to those found for the well characterized association of that proportion of erythrocyte band 3 which is associated with the cytoskeleton [11].

The above results suggest that the Rh(D) polypeptide may be linked to the human erythrocyte cytoskeleton. This transmembrane polypeptide could form an attachment site for the skeletal matrix with the membrane. This would be additional to the well characterised interaction between ankyrin and band 3 [7] and the possible interaction between the cytoskeleton and glycoconnectin [4,12]. Estimates of the number of Rh(D) antigen sites in cDE and -D- cells have yielded values of approx. 40000 and 150000 sites per cell, respectively [8]. Thus, the potential number of membrane attachment sites in -D- cells provided by the Rh(D) polypeptide would be similar in magnitude to the number of band 3 molecules bound to ankyrin [7].

Table 1

Phospholipid content of cytoskeleton preparations

Triton X-100 (%)	% ghost phospholipid retained	
	150 mM NaCl	10 mM Tris–Cl
1	32 ± 12 (3)	20 ± 5 (3)
5	14 ± 5 (2)	9 ± 3 (3)
10	6.5 ± 1 (2)	5 ± 2 (3)

Results are means ± SD, with the number of determinations in parentheses

The erythrocyte cytoskeleton is believed to play an important role in maintaining the shape and integrity of the red cell membrane. There are known associations between altered erythrocyte shape and the Rh blood group system. Rh_{null} erythrocytes have a total lack of all antigens within the Rhesus blood group system and have an abnormal shape (stomatocytosis). The condition is associated with mild chronic haemolytic anaemia and the cells have an increased osmotic fragility [9]. These cells appear to lack two extracellular sulphydryl-group-containing polypeptides, one of which is identical to the Rh(D) polypeptide [5]. There is also a genetic linkage between the Rh blood group system and certain forms of elliptocytosis [10].

These observations together with the present results suggest that the Rh(D) polypeptide may play an important functional role in the maintenance of cell shape and viability in the erythrocyte.

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