Biochimica et Biophysica Acta, 687 (1982) 97–100
Elsevier Biomedical Press

BBA Report

BBA 70027

IDENTIFICATION OF ECTOPROTEINS OF HUMAN PLATELETS

II. PROTEINS OF LOW MOLECULAR WEIGHT (10000–43000)

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(Received November 13th, 1981)

Key words *Ectoprotein, Cell surface, Membrane glycoprotein, Two-dimensional electrophoresis, Radioactive labeling, (Human platelet)*

The surface exposed proteins in the range of 43 to 10 kDa were studied with a combination of radiolabelling and two-dimensional electrophoresis. Nineteen proteins were found after reduction, nine of which were linked to other polypeptides in the non-reduced state.

Membrane ectoproteins are involved in essential platelet activities, such as adhesion and aggregation. In Glanzmann's thrombasthenia aggregation is defective and two glycoproteins (IIb and III) are diminished or absent [1]. Adhesion of blood platelet to subendothelium is disturbed in the Bernard-Soulier syndrome [2]. This adhesion is mediated by factor VIII-VWF factor [3,4] and Bernard-Soulier platelets possess decreased quantities of glycoprotein Ib [5,6] the putative receptor for factor VIII-VWF [7]. The use of a combination of radiolabelling techniques with two-dimensional high resolution electrophoresis [8,9] has recently allowed identification of about 25 surface exposed membrane proteins in the range of 30–300 kDa [10]. That study as well as investigation of inherited disorders concentrated on proteins above 30 kDa and provided no information on proteins of lower molecular weight.

In the present paper we describe the ectoproteins in the range of 10–43 kDa studied by three different radioactive labelling techniques.

Radioactive labelling of human blood platelets by lactoperoxidase labelling [11], [^{125}I]iodosulfanilic acid labelling and periodate boro[^{3}H]hydride labelling [12] was performed as previously described [10]. Two-dimensional electrophoresis using isoelectric focussing in the first dimension [8] as modifed by Clemetson [13] was performed as previously described [10], but a 12.5% polyacrylamide slab gel (2.6% cross-linking) was used with a 1 cm 3% stacking gel (2.6% cross-linking). Two-dimensional electrophoresis of non-reduced samples in the first dimension followed by reduction and electrophoresis in the second dimension (non-reduced/reduced) [9] was performed as previously described [10], but with a 12.5% gel in both dimensions.

The patterns obtained with the three labelling techniques are sufficiently similar to warrant a combined discussion. Fig. 1 shows the autoradiogram of a non-reduced/reduced gel after lactoperoxidase labelling, and Fig. 2 shows a compound diagram based on data from all three labelling methods. The specific details of the various proteins are summarized in Table I.

An autoradiogram of reduced proteins labelled

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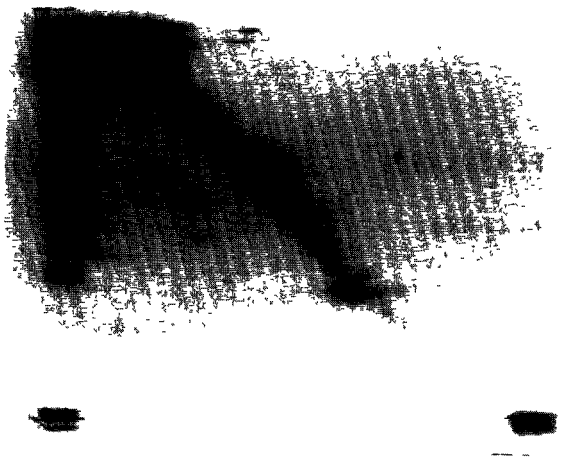

Fig 1 Autoradiogram of membrane proteins exposed on the surface labelled with the lactoperoxidase technique Platelets were treated with SDS-*N*-ethylmaleimide and subjected to two-dimensional electrophoresis of non-reduced/reduced proteins according to Ref. 9 with 12 5% polyacrylamide in both dimensions For markers see Fig 2

with iodosulfanilic acid and subjected to two-dimensional electrophoresis with isoelectric focussing in the first dimension is shown in Fig. 3. The compound diagram based on the results of all

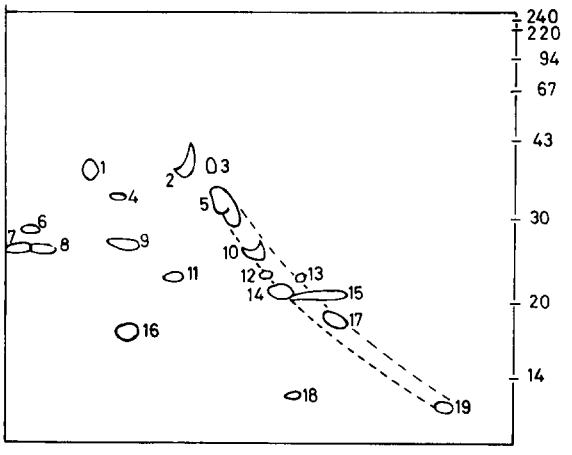

Fig 2 Composite diagram of two-dimensional electrophoresis of non-reduced proteins in the first dimension and after reduction in the second dimension The diagram is based on data obtained with lactoperoxidase, periodate-borohydride and iodosulfanilic acid labelling. Markers used were spectrin 240 kDa and 220 kDa, phosphorylase B 94 kDa bovine albumin 67 kDa, ovalbumin 43 kDa, carbonic anhydrase 30 kDa, soy bean trypsin inhibitor 30 kDa and α-lactalbumin 14 kDa.

TABLE I

CHARACTERISTICS OF LABELLED SURFACE PROTEINS

$M_{r,app}$, apparent molecular weight, reduced, LP, lactoperoxidase, BM, borohydride, ISA, iodosulfanilic acid

Number in Fig 2	$M_{r,app}$ ($\times 10^{-3}$)	LP	BH	ISA	General remarks
1	36	+	+	+	From 85 kDa by reduction Coomassie blue positive
2	43	++	+	+	Heavy chain of HLA? Often deplaced by actin to lower mol wt
3	38	++	++	++	Intrachain disulfide bridge
4	33	+	+	+	From 67 kDa together with 9 and 16 Coomassie blue positive
5	31	++	+	+	
6	28	±	+	+	Icβ
7	23	+	++	++	Ibβ Coomassie blue positive
8	23	+	++	++	IIbβ Coomassie blue positive
9	23	+	+	+	From 67 kDa together with 4 and 6 Coomassie blue positive
10	26	+	+	+	
11	22	+	+	+	From 46 kDa
12	22	+	+	+	
13	22	+	−	−	
14	21	++	++	++	Coomassie blue positive Slight decrease in mol wt on reduction
15	21	±	±	±	
16	16	−	−	+	From 67 kDa together with 4 and 9 Coomassie blue positive
17	18	±	+	++	
18	13	+	−	+	From 21 kDa
19	12	+	−	+	β_2 microglobulin

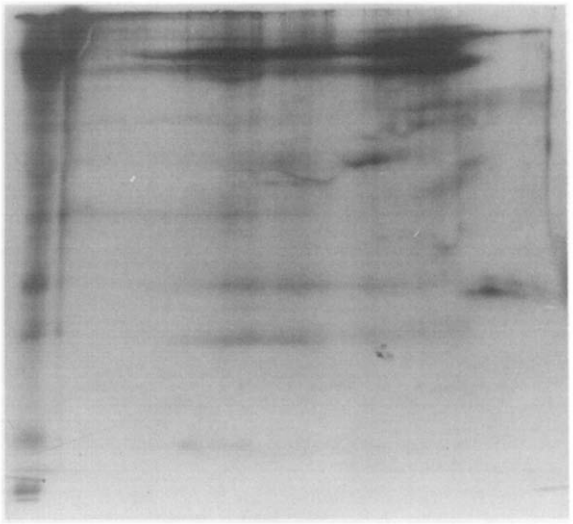

Fig 3 Autoradiogram of membrane proteins exposed on the surface labelled with the iodosulfanilic acid technique Platelets were treated with SDS-β-mercaptoethanol and subjected to two-dimensional electrophoresis with isoelectric focussing, pH range 7 4–5 2, in the first dimension using 12 5% polyacrylamide For markers see Fig 2

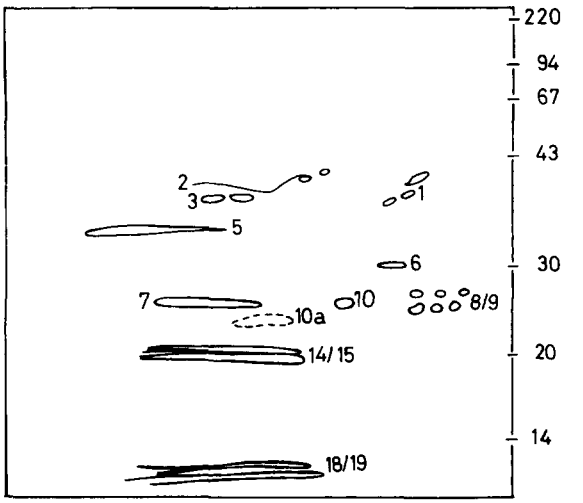

Fig 4 Composition diagram of two-dimensional electrophoresis with isoelectric focussing, pH range 7 4–5.2, in the first dimension The diagram is based on data obtained with lactoperoxidase, periodate-borohydride and iodosulfanilic acid labelling For markers see Fig 2

three techniques is shown in Fig. 4. The spots in the range of 25–43 kDa of Figs. 3 and 4 agree well with those described in region IV of Fig. 2 of our previous paper [10].

Protein 16 from Fig. 2 was only seen with iodosulfanilic acid labelling and proteins 17, 18, and 19 were not seen with borohydride labelling. A problem arises with the numbering of 7, 8, 9 and 10 in the two-dimensional gel with isoelectric focussing as compared to the non-reduced/reduced gel. Direct studies of non-reduced two-dimensional gels with isoelectric focussing in the first dimension (not shown) indicate that both protein 10 and one protein in the 8/9 area do not change on reduction. Only one spot of this molecular weight (10) appears on the diagonal in non-reduced/reduced gels but this may represent two different proteins.

The proteins 6, 7 and 8 correspond to the subunits Icβ [6], Ibβ [7], and IIbβ originating from major glycoproteins [9]. In the two-dimensional gel with isoelectric focussing glycoprotein Ibβ has a basic pH as was reported by Clemetson et al. [14]. The proteins 4, and 16 originate probably from a single protein with an apparent molecular weight of around 68 kDa. The only possible candidate in this region is protein 20 from Fig. 5 in Ref. 12. No possible candidate was found in that figure for the 85 kDa protein from which protein 1 is derived. Protein 19 probably represents β_2 microglobulin on the basis of its apparent molecular weight of 12 kDa which does not change on reduction and its lack of terminal sialic acid as evidenced by the absence of labelling with the borohydride technique [15].

Several of the protein observed on the non-reduced/reduced gels were not found on the two-dimensional gel using isoelectric focussing. The most probable reason for this is that their isoelectric point falls outside the range of 5.4–7.2, which we have used for these studies. Extending the pH range to the acidic side towards a pH of 4.0 demonstrated no new proteins which indicates that such proteins may be basic. It should be mentioned that the identification of proteins comparing both two-dimensional techniques is sometimes of necessity tentative when several proteins are in the same molecular weight range (e.g. No. 7–10).

The advantage of the use of two-dimensional techniques [8,9] is that they combine high resolu-

tion with reproducibility. Extension of this technique to the lower molecular weight region allowed us to recognise a number of previously unidentified platelet ectoproteins. Such knowledge is of importance as a frame of reference for the identification of specific proteins involved in disease or function.

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