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INTERACTION BETWEEN ERYTHROCYTE MEMBRANE PROTEINS AND COMPLEMENT COMPONENTS

I. THE ROLE OF -S-S LINKAGES AS REVEALED BY TWO-DIMENSIONAL SODIUM DODECYLSULFATE–POLYACRYLAMIDE GEL ELECTROPHORESIS

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SUMMARY

1. The peptide patterns of sheep erythrocyte membranes treated with antibody and inactivated/activated complement show characteristic differences in sodium dodecylsulfate–polyacrylamide gel electrophoresis. When active complement is used, new peptide bands of high molecular weight are observed. These are split into peptides of lower molecular weight upon treatment with dithiothreitol.

2. The importance of disulfide bonds can be demonstrated by two-dimensional sodium dodecylsulfate–polyacrylamide gel electrophoresis. Labelling of membrane proteins with dansyl-chloride prior to treatment with antibody and complement allows differentiation between membrane and non-membrane proteins. We can thereby demonstrate that following complement lysis, protein complexes exist in sodium dodecylsulfate which contain both membrane and non-membrane proteins.

3. Blockage of membrane sulfhydryl groups with parachloromercuribenzoic acid (sodium salt) prior to treatment of membranes with antibody and complement does not alter the two-dimensional electrophoretic pattern. We suspect that the non-membrane proteins are complement components, some of which are directly linked to membrane proteins. There is no evidence for other than non-covalent bonds between these proteins.

4. Experiments utilizing normal and C6-deficient rabbit sera indicate that the main non-membrane proteins visualized by two-dimensional sodium dodecylsulfate–polyacrylamide gel electrophoresis are early acting components of the complement system. Data are presented which suggest that one or more of these components penetrates into the hydrophobic membrane interior, thereby effecting attachment to

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membrane proteins. Minor peptides which may represent some of the late acting components can also be observed.

INTRODUCTION

Numerous investigations carried out in the last decade have led to the delineation of the main biochemical changes undergone by complement proteins during the process of their activation and assembly on target cell surfaces [1–3]. However, we do not know how complement ultimately lyses membranes. The concept of phospholipid catabolism as the critical step toward complement-mediated cell lysis has been pursued by many investigators, but no well-defined and reproducible changes have been found amongst these membrane components [4–8].

Studies on liposomal systems have led Kinsky to suggest that membrane perturbation may be the result of detergent action manifested by the activated components themselves [9]. Finally, a recently proposed hypothesis of complement action suggests that complement components might form hydrophilic channels penetrating through membranes, thereby producing membrane leakiness [10].

One approach to the understanding of lytic complement mechanism is the analysis of complement proteins at the membrane level, but few attempts have been made at analysing membrane and membrane-bound proteins in connection with complement action. Such investigations would serve two purposes. First, they would allow one to detect possible biochemical changes in membrane proteins. Second, such analyses might shed light on the molecular nature of membrane-bound complement, and thus contribute to a better understanding of complement action.

We are exploring both possibilities and in this paper present results of detergent gel electrophoretic analyses of membranes following complement lysis. In a following communication we shall demonstrate the separation and partial electrophoretic characterisation of complement components desorbed from the membranes and their subsequent electrophoretic analyses.

MATERIALS AND METHODS

We have obtained all chemicals and biochemicals from Serva (Heidelberg), Boehringer (Mannheim) and Merck (Darmstadt). We obtained fresh human serum from the Freiburg Blood Bank. U. and K. Rother kindly supplied us with serum of C6-deficient and normal rabbits [11].

Membrane preparation. We wash sheep erythrocytes five times with isotonic saline, taking care to remove the buffy coat, and prepare membranes as in [12]. After 3–4 washings with 5 mM phosphate buffer, pH 8.0, we obtain faintly pink membranes which we use in experiments utilizing isolated membranes treated with antibody and complement. Ghost protein concentration is determined with the Ninhydrin method [13].

Treatment of cells and isolated membranes with antibody and complement. We suspend washed erythrocytes to a concentration of 10^9 cells/ml in veronal buffered saline 1.0 mM in Mg^{2+} and 0.15 mM in Ca^{2+}. To one vol. of a cell suspension

we add one vol. of rabbit anti-sheep red blood cell serum (antibody titer 1600) diluted with 50 vols of buffer. Following 15 min's incubation at 37 °C, we wash the antibody-coated cells once in the same buffer and resuspend to 10^9 cells/ml, add one-tenth vol. of fresh human serum or one-fifth vol. of C6-deficient or normal rabbit serum to the cells and allow complement reaction to proceed at 37 °C. With normal sera, lysis is complete within 7 min, whereas no lysis takes place when C6-deficient serum is used. We centrifuge cells treated with C6-deficient serum at 4 °C and wash these cells four times with ice-cold saline. Electrophoretic examination of the supernatant fluid of the last wash shows that all non-membrane-bound proteins have been removed. We then lyse the cells with 5 mM phosphate buffer treated pellet membranes by centrifuging at $20\,000 \times g$ for 10 min in a Sorvall centrifuge model RC2-B and thereafter wash four times with ice-cold 5 mM phosphate, pH 8.0.

When using membranes as targets, we suspend these (approximately 10^9 ghosts/ml) in veronal-buffered saline and treat them with antibody and complement as in the case of cells. Then our controls are untreated ghosts, membranes coated with antibody alone and antibody-coated membranes treated with heat-inactivated complement (1 h at 56 °C).

Labelling of membrane proteins. We label erythrocytes and isolated membranes with 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl-chloride) as in [14]. Cell lysis with antibody and complement proceeds in an unchanged fashion when dansylated erythrocytes are employed as targets.

Blockage of membrane-SH groups. We suspend isolated membranes in veronal-buffered saline to a concentration of approximately 10^9 membranes/ml, make this suspension 10^{-3} M in *p*-chloromercuribenzoic acid (sodium salt), incubate at room temperature for 60 min, subsequently wash membranes five times with 5 mM phosphate buffer and following this, incubate the membranes with antibody and complement as outlined above.

In order to determine the extent of –SH blockage with *p*-chloromercuribenzoic acid, we have performed titrations of membrane –SH groups with Ellman's reagent (dithiobisnitrobenzol) [15]. For this, we solubilize washed membranes in 3 % sodium dodecylsulfate and 0.1 M phosphate buffer, pH 8.0, and subsequently titrate –SH groups as in [16] using cysteine as a standard. With this method, approximately $3.0 \cdot 10^{-17}$ moles –SH groups are detected per white ghost. Following treatment of membranes with *p*-chloromercuribenzoate, no –SH groups are reactable with Ellman's reagent in sodium dodecylsulfate, indicating that all –SH groups titrable with this reagent have been reacted.

Membrane solubilisation. We suspend washed membranes to a concentration of approximately 4 mg membrane protein/ml in 5 mM phosphate, add sodium dodecylsulfate to a final concentration of 4 %, heat (100 °C) for 3 min, add dithiothreitol to a final concentration of 20 mM to aliquots of all samples and incubate these for 10 min at 37 °C.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis. We proceed essentially as in [17], employing 5 % acrylamide gels cross-linked to 2.5 % with *N,N'*-methylenebisacrylamide. We use gels 0.5 cm in diameter and 10 cm in height for one-directional runs, and gels 0.6 cm in diameter and 15 cm in height for two-dimensional studies. The electrophoresis buffer is 0.04 M Tris–HCl, 0.02 M sodium acetate, 0.002 M EDTA, pH 7.4, containing 1 % sodium dodecylsulfate. Electrophoresis in short gels

is at 5 mA/gel for about 2 h and in long gels at 1.5 mA/gel overnight at room temperature, until the tracking dye, Pyronin G, has migrated 7 cm and 14 cm, respectively.

Two-dimensional sodium dodecylsulfate–polyacrylamide gel electrophoresis. We proceed essentially as in [18] with minor apparatus modifications for use with sodium dodecylsulfate (Knüfermann, H., Bhakdi, S., and Wallach, D. F. H., in preparation). We use 20 × 20 × 0.5 cm gel slabs with an acrylamide concentration of 5.0 %, cross-linked to 2.5 % with *N,N'*-methylenebisacrylamide. We run sodium dodecylsulfate-electrophoreses at 100 mA per gel slab at room temperature for about 6 h. The migration distance of the tracking dye in the second dimension is about 13 cm. We use the same electrophoresis buffer as in the unidirectional system, but add sodium dodecylsulfate to the upper cathodal buffer only.

Determination of molecular weights. We calibrate gels as in [19, 20] using rabbit immunoglobulin G (155 000), phosphorylase A (93 000), bovine serum albumin (69 000), egg white albumin (45 000), bovine pancreas α-chymotrypsinogen A (25 700), horse skeletal muscle myoglobin (17 200) and horse heart cytochrom *l.c.* (13 500) as standard proteins.

Experimental protocol. For two-dimensional studies, we electrophorese 100 µl aliquots containing approximately 400 µg of membrane protein solubilized in sodium dodecylsulfate, allow the tracking dye to migrate 14 cm, then extract the gels from the glass tubes and immediately dialyse these against electrophoresis buffer containing 1 % sodium dodecylsulfate and 20 mM dithiothreitol for 1 h at room temperature. We subsequently polymerize the gels horizontally onto gel slabs [18] and proceed with electrophoresis in the second dimension.

Staining and evaluation procedures. We stain gel columns and slabs with Coomassie Brilliant Blue according to [17], and perform densitometric scanning of stained gel columns in a Gilford spectrophotometer (model 240) with a linear transport (model 2410-5). Following two-dimensional electrophoresis of membranes prelabelled with dansyl-chloride, we view gel slabs under ultraviolet light directly after running in the second dimension. We mark fluorescing spots by injecting these with trace amounts of India ink and subsequently stain with Coomassie Blue.

Photography of gels. We photograph discs and slabs with a Polaroid Land MP-3 camera using a Polaroid film type PN 55. Shutter settings are 11/16 and 16/22 for gel discs and slabs, respectively. Exposure time is 1 s.

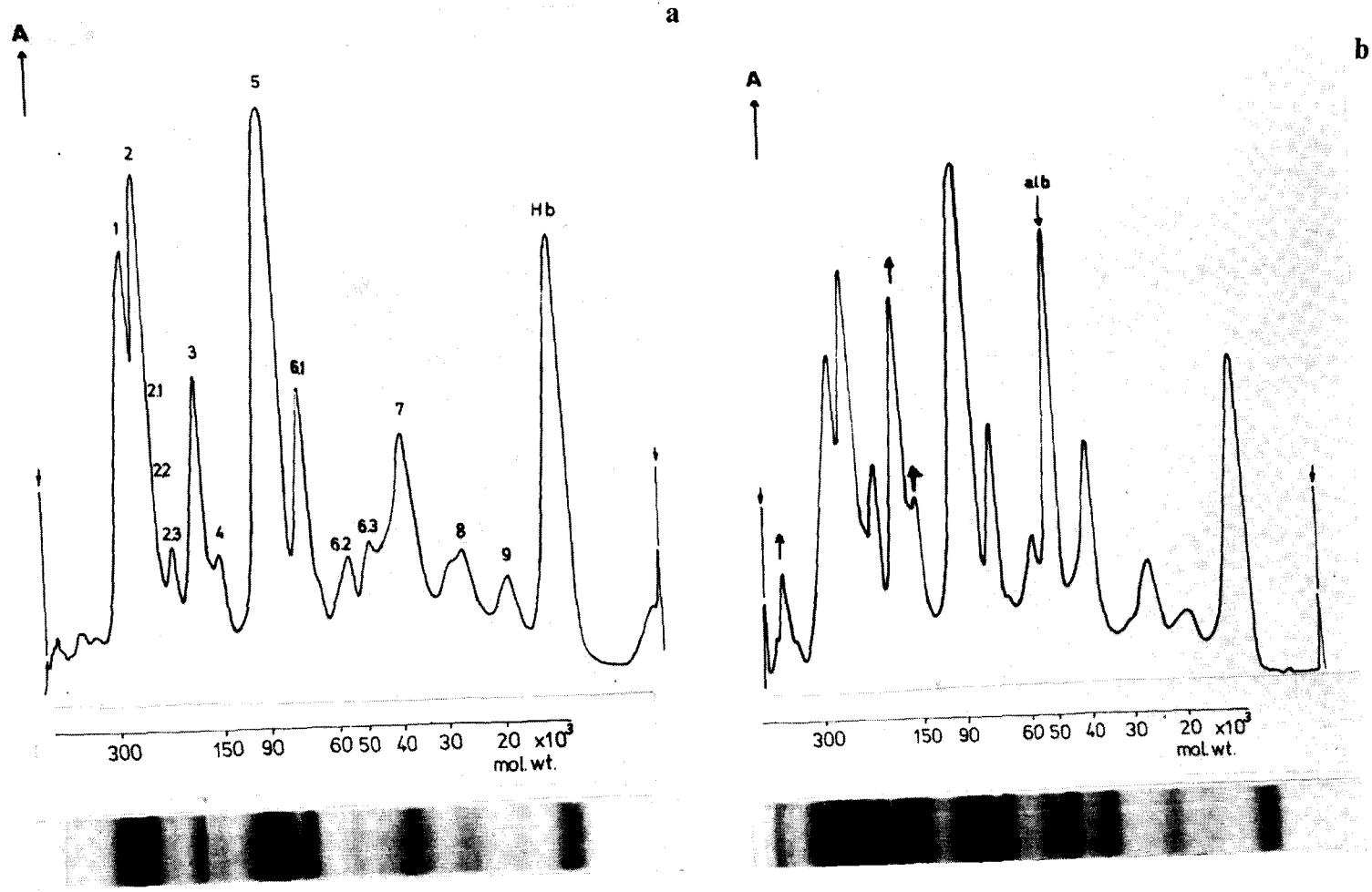
ESULTS

Sodium dodecylsulfate–polyacrylamide gel electrophoresis of membrane proteins

Fig. 1 depicts the sodium dodecylsulfate–polyacrylamide gel electrophoresis patterns and the corresponding scans of erythrocyte membranes treated with antibody and activated/inactivated human complement.

(a) *The normal peptide pattern of sheep erythrocyte membranes.* We have numbered the peptide bands 1–9 in order of decreasing apparent molecular weight. Addition of dithiothreitol to the solubilized sample causes no discernible change in the pattern, indicating the absence of intermolecular disulfide bonds amongst the major membrane proteins.

(b) *Membranes treated with antibody and inactivated complement.* Planimetric quantitation of scans show that there is an increase in staining intensity in the regions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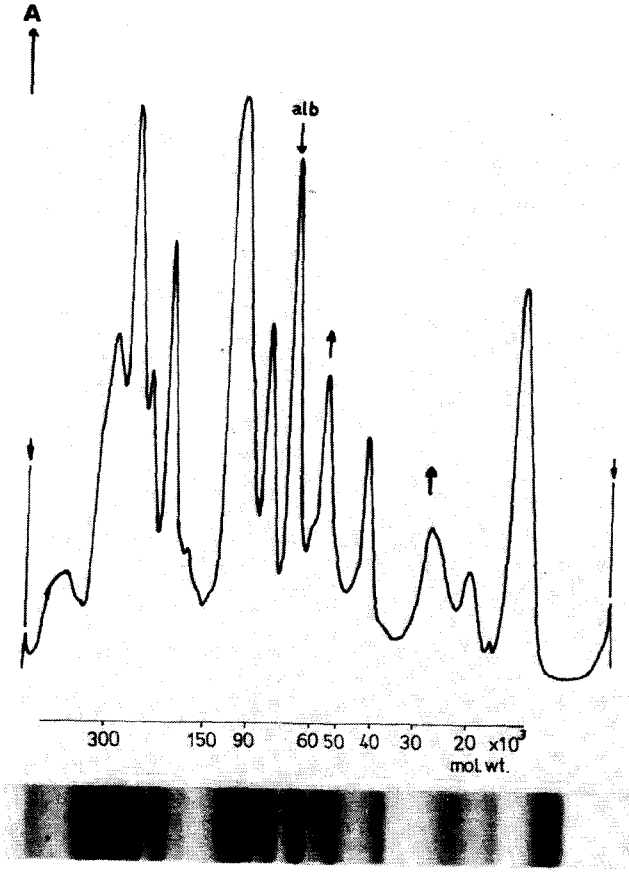
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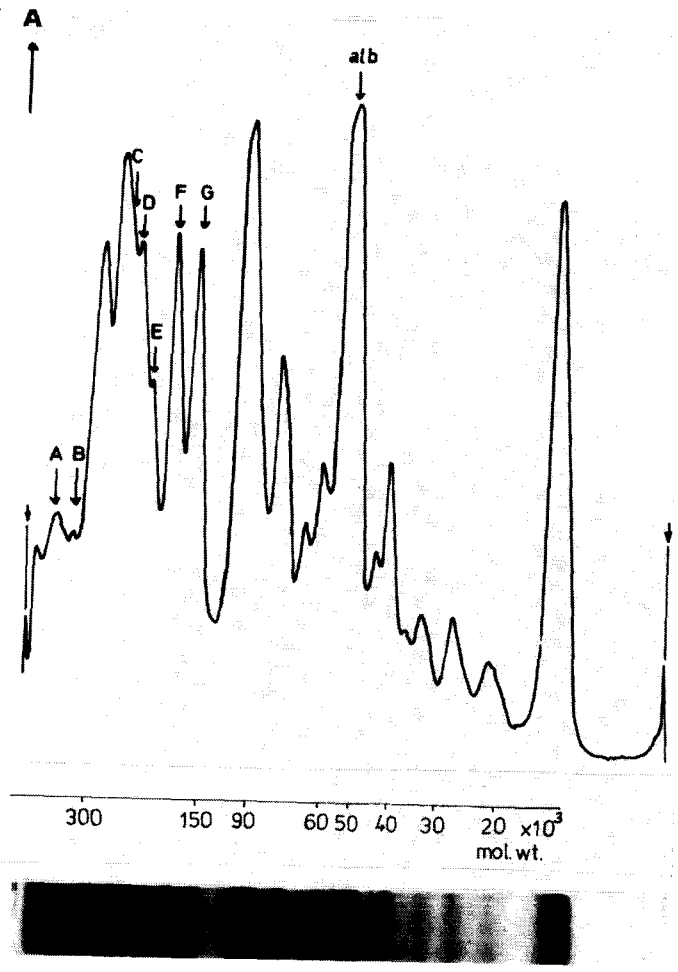
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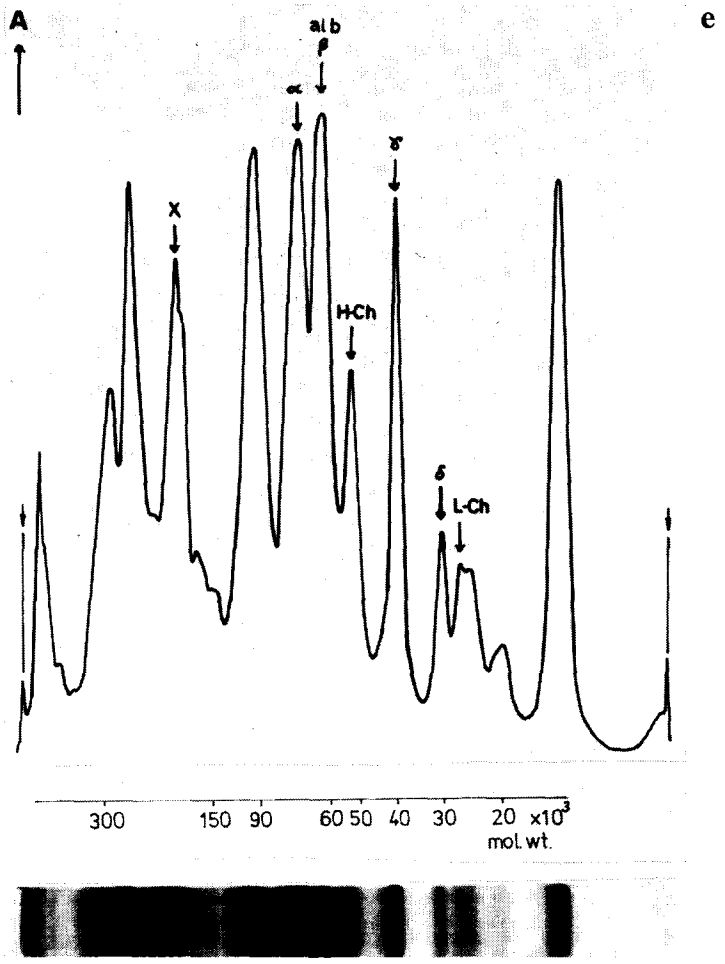

Fig. 1. Changes in the peptide patterns of sheep erythrocyte membranes following complement lysis. Sodium dodecylsulfate–polyacrylamide gel electrophoresis patterns and the corresponding scans. A, absorbance at 576 nm. (a), normal peptide pattern of sheep erythrocyte membranes. Hb, hemoglobin. (b) and (c), membranes treated with antibody and heat-inactivated human complement. alb, albumin. (d) and (e), membranes treated with antibody and human complement. Dithiothreitol added to samples (c) and (e). alb, albumin; ↑: Increased staining intensity with Coomassie Brilliant Blue. Details in text. H-Ch, heavy chain; L-Ch, light chain.

of protein bands 2.3, 3, 4 (150–170 000 daltons), 6.3 (60 000 daltons) and also in the high molecular size region of approximately 1 000 000 daltons.

(c) *Membranes treated with antibody and inactivated complement; dithiothreitol added to solubilized sample.* After disulfide cleavage, the increased staining intensity in the regions of bands 3 and 4 (corresponding to 150–170 000 daltons) is no longer discernible. Instead, staining intensity in the molecular weight region of 55 000 and 27 000 increases. The band seen in gel (b) at the molecular weight region of membrane protein 6.3 (approx. 55 000) appears to shift to a slightly higher molecular weight region (approx. 65 000) but remains unchanged in intensity. This protein

band is absent when *intact* cells are treated with antibody and inactivated complement. The protein has the same molecular weight and shows the same electrophoretic behaviour as serum albumin, and is also found when isolated membranes are incubated in a solution of pure albumin. It thus appears that albumin attaches itself non-specifically to isolated membranes under our experimental conditions, and is not removed by washing with 5 mM phosphate buffer.

(d) *Peptide pattern of membranes treated with antibody and active complement.* Intact cells lysed with complement or isolated membranes treated with antibody and complement give completely identical peptide patterns. Labelling of intact cells or isolated membranes with dansyl-chloride prior to treatment with antibody and complement does not affect the electrophoretic pattern. A series of new bands, all of apparent molecular weight $> 100\,000$ can be seen, which we arbitrarily name bands A–G. Some of these bands are superimposed on membrane protein bands and lead to an increase in staining intensity in the corresponding regions.

(e) *Membranes treated with antibody and complement; dithiothreitol added to solubilized sample.* Upon disulfide cleavage all but one of the new bands disappear. We name the remaining high molecular weight band "X". This has a relative mobility close to but slightly lower than that of membrane protein 3, and occupies a position immediately behind this membrane component. Coomassie Blue-staining intensity increases in the molecular weight region of approximately 80 000, 70 000, 45 000 and 30 000, and we arbitrarily name these enhanced bands α, β, γ and δ respectively.

Fig. 2. Sodium dodecylsulfate–polyacrylamide gel electrophoretic patterns of sheep erythrocyte membrane proteins treated with rabbit complement. Electrophoretic run: top to bottom. (a) and (b), membranes treated with antibody and C6-deficient serum. (c) and (d), membranes treated with antibody and normal rabbit serum. Dithiothreitol added to samples (b) and (d).

Fig. 2 shows the peptide patterns obtained when sheep erythrocyte membranes are reacted with antibody and C6-deficient (gels a and b) or normal rabbit complement (gels c and d). When intact cells are reacted with antibody and C6-deficient serum, the albumin band is absent. The peptide patterns are similar, but not identical to those obtained with human serum. No differences can be discerned between the patterns obtained with C6-deficient and normal rabbit serum in this one-dimensional system.

Two-dimensional sodium dodecylsulfate gel electrophoresis

The difficulty in interpreting any findings on the basis of unidirectional detergent gel electrophoresis alone is that one cannot distinguish between two basically different proteins which have similar or identical molecular weights. Thus, for example, an increase in staining intensity of peptide 7 after reduction of solubilized complement-treated membranes (Fig. 1e) suggests but cannot prove that a new protein not identical with membrane protein 7 is superimposed on this band. Therefore, a two-dimensional separation technique is indispensable for an exact correlation between the electrophoretic patterns obtained with and without disulfide cleavage. In this, we cleave disulfide bonds with dithiothreitol between first and second dimensional runs in an otherwise identical electrophoretic system.

When erythrocyte membranes are electrophoresed in this manner, we see only a diagonal of peptide spots on the two-dimensional plate, because none of the membrane peptides are affected by dithiothreitol treatment. Fig. 3 demonstrates the

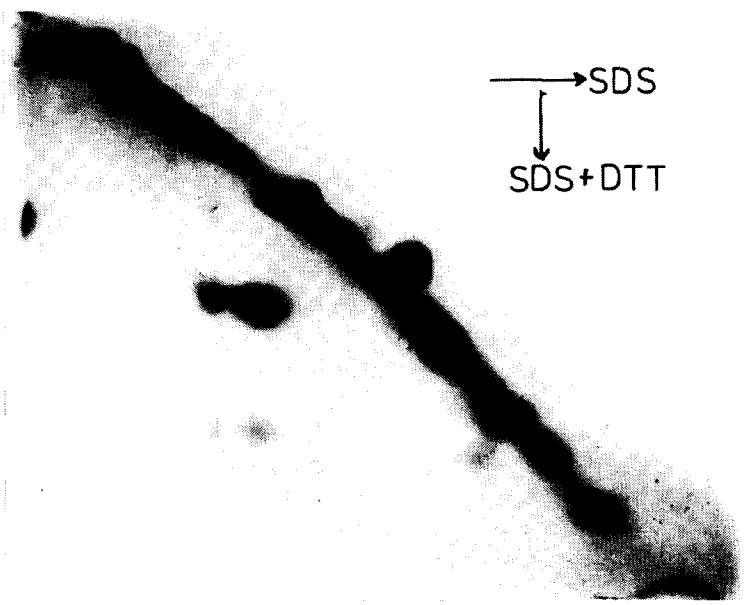

Fig. 3. Two-dimensional sodium dodecylsulfate–polyacrylamide gel electrophoresis of erythrocyte membranes treated with antibody and inactivated human complement. Cleavage of disulfide bonds between first and second-dimensional runs. First dimension: left to right, second dimension: top to bottom. SDS, sodium dodecylsulfate; DTT, dithiothreitol.

separation of peptides of membranes treated with antibody and inactivated complement. The diagonal peptide spots represent membrane proteins, but the protein of molecular weight 150 000–170 000, splitting into two peptides of molecular weight 55 000 and 27 000 after dithiothreitol, is most likely membrane-bound immunoglobulin. Another molecular weight protein splits into peptides of molecular weights 90 000, 55 000 and 27 000 after dithiothreitol. Finally, the peptide corresponding electrophoretically to albumin shows decreased electrophoretic mobility after treatment with dithiothreitol, resulting in the displacement of the peptide above the diagonal line.

The two-dimensional separation allows one to retrospectively explain the one-dimensional electrophoretic findings described above. Thus, the appearance of new, Coomassie Blue stainable material in the molecular size region of membrane proteins 3 and 4 (150 000–170 000 daltons) after reaction of membranes with antibody can only suggest that this represents membrane-bound immunoglobulins (Fig. 1b). In uni-directional electrophoresis, disulfide reduction will cause disappearance of this material and appearance of new material in the molecular weight region of 55 000 and 27 000 (Fig. 1c). However, detailed planimetric quantitation is needed to clearly demonstrate the 27 000 dalton component, because this stains lightly and is superimposed upon membrane protein 8. On the other hand, in two-dimensional electrophoresis the two components of the 150–170 000 dalton protein generated by dithiothreitol become very conspicuous because of the absence of other protein spots in their vicinity (Fig. 3), and it is apparent that the new peptide spot at 27 000 daltons cannot represent membrane protein 8. The contention that we are observing cleavage of immunoglobulins into heavy and light chains thus receives strong support. Moreover, two-dimensional electrophoresis shows that there are two molecular species of membrane-bound immunoglobulins having slightly different molecular weights corresponding to those of membrane proteins 3 and 4. This explains why in unidirectional electrophoresis the staining intensity of both these bands increases (fig. 1b). Analogously, the displacement of albumin above the diagonal line of protein spots, demonstrating decrease in relative mobility after dithiothreitol treatment, proves the contention that the corresponding bands seen in unidirectional electrophoresis (Fig. 1 b and c) represent one and the same protein, again not identical to any membrane protein.

Fig. 4a shows a two-dimensional separation pattern of complement-treated membranes. Dansylation of membrane proteins prior to treatment with antibody and complement allows differentiation between membrane proteins and non-membrane peptides. In Fig. 4b we present a schematic illustration of the same gel slab together with the results of the dansylation experiment. The corresponding unidirectional gel patterns are also presented schematically. Fluorescing components have been marked black. Dotted spots indicate non-membrane peptides which are attached independent of complement action, i.e. immunoglobulins and serum albumin. Empty circles represent non-fluorescing peptides whose appearance is directly related to and dependent on complement action. When varying concentrations of complement are used, these non-fluorescing spots vary accordingly in intensity.

Each of the high molecular weight complexes termed A, C, D and E gives rise to one fluorescing peptide and a series of three non-fluorescing peptides, whose electrophoretic mobility correspond to peptides α, γ, δ in the unidirectional system.

Bands B, F and G, on the other hand, do not generate fluorescing spots. Complex B gives rise to two high molecular weight proteins (apparent molecular weight about 180 000) and three lower molecular weight peptides of apparent molecular weight 80 000, 45 000 and 30 000. The high molecular weight components contribute toward the formation of peptide X, whereas the lower molecular weight components make up part of bands α, γ, δ in the one-dimensional gel patterns (Fig. 1h). Complex F yields four non-fluorescing spots corresponding to bands α, β, γ, δ in one-dimensional electrophoresis. Complex G gives rise to one major peptide of decreased electrophoretic mobility which comprises the major part of band X (Fig. 1, gel h), plus two minor peptides of molecular weight 80 000 and 45 000. Here it again becomes apparent that protein X is not identical to membrane protein 3, although in unidirectional electrophoresis separation is sometimes not completely clear because these two proteins migrate almost identically (Fig. 1e). Superimposed upon the line of peptide spots generated by complex G are the heavy and light chains of IgG, because IgG migrates in the same region as complex G in the first dimensional run. Finally, two to three minor, non-fluorescing peptides of molecular weight 90 000–95 000 are constantly seen. They are not in line with the major bands A–G, and are thus probably independent entities which, however, have also been affected by dithiothreitol treatment.

Results of parachloromercuribenzoic acid treatment of membranes

Blockage of sulfhydryl groups of membrane proteins prior to addition of antibody and complement does not result in any change of the peptide pattern. We have also used N-ethylmaleimide, Ellman's reagent (dithiobisnitrobenzol) and iodoacetamide to block membrane sulfhydryl groups with no difference in our electrophoretic results.

Normal and C6-deficient rabbit complement

Fig. 5a shows a two-dimensional pattern of membrane proteins following treatment with rabbit complement. Fig. 5a is a schematic illustration of the gel slab together with the results of experiments utilizing C6-deficient sera. The spots marked black in this illustration are not seen when C6-deficient serum is used. They correspond to an apparent molecular weight of around 95 000 and represent very small amounts of material. They escape detection in the one-dimensional system, but can always be seen in the two-dimensional plates. Importantly, the main non-fluorescing spots, representing peptides complexed to membrane proteins are observed also when intact erythrocytes are treated with C6-deficient serum.

DISCUSSION

After reacting active complement with dansylated erythrocyte ghosts, sodium dodecylsulfate–polyacrylamide gel electrophoresis in one dimension followed by sodium dodecylsulfate–polyacrylamide gel electrophoresis in dithiothreitol at right angles reveals several non-fluorescent peptide components (empty circles in Fig. 4b). These do not appear when inactivated complement is used.

The intensity of these components varies directly with the concentration of complement. They are generated from high molecular weight complexes when these are treated with a disulfide-splitting reagent such as dithiothreitol. In contrast,

membrane proteins are not affected by the action of dithiothreitol.

The membrane peptide pattern appears basically unchanged after complement action, as can be seen by analysis of the diagonal representing membrane proteins in the two-dimensional separation plate (Figs. 4 and 5). This supports the contention that the non-fluorescing spots represent non-membrane proteins; we shall henceforth refer to them as complement proteins. More direct evidence for the complement origin of these peptides will be presented in a further communication.

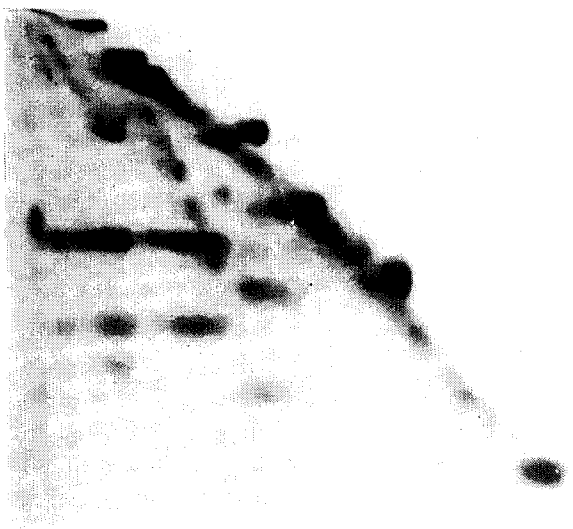

Fig. 4a. Two-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis of erythrocyte membrane proteins treated with antibody and human complement. Disulfide cleavage between first and second dimensional runs. First dimension: left to right, second dimension: top to bottom.

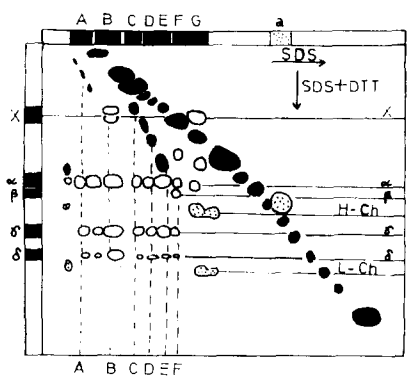

Fig. 4b. Schematic illustration of the gel plate in Fig. 4a, giving the results of the dansylation experiment (details in text). Black spots: fluorescing peptides, dotted circles: non-membrane peptides which are attached to the membrane independent of complement action, empty circles: non-fluorescing peptides whose appearance is directly related to complement action. a, albumin; SDS, sodium dodecylsulfate; DTT, dithiothreitol; H-Ch, heavy chain; L-Ch, light chain.

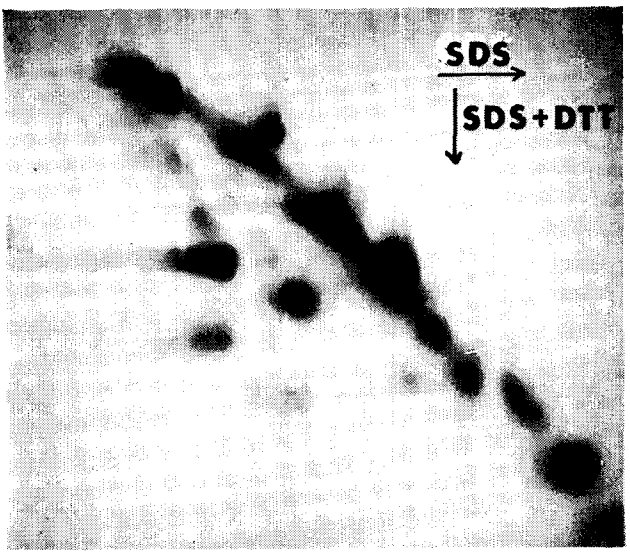

Fig. 5a. Two-dimensional sodium dodecylsulfate–polyacrylamide gel electrophoresis of membranes treated with antibody and rabbit complement. SDS, sodium dodecylsulfate; DTT, dithiothreitol.

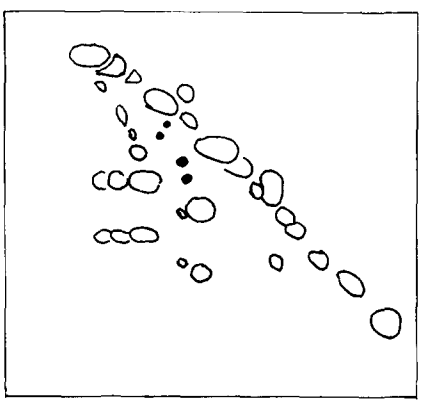

Fig. 5b. Schematic illustration of gel plate in Fig. 5a giving the results of experiments utilizing C6-deficient sera. Black spots: peptides absent with C6-deficient serum.

Our data show that complement components earlier than C6 can be readily detected in our detergent-gel electrophoresis system. Erythrocyte membranes retain only traces of later reacting components, even when a great excess of antibody and complement is applied.

Our experimental approach clearly demonstrates the existence of a multitude of disulfide bonds amongst complement proteins. The molecular structure of native complement proteins is under active investigation in many laboratories, and intramolecular disulfide bonds have been shown to exist in components 1 [21], 3 [22] and 5 [23] of the complement system. However, detailed information on their molecular composition is still scarce, and no data have been reported previously concerning the biochemistry of membrane-bound complement components. We cannot at present

identify the various complement spots which we visualize, but this can be achieved in future experiments utilizing radiolabelled complement components and/or with immune-fixation techniques.

Interestingly, one or several of the earlier acting components remain associated with membrane proteins even after membrane solubilisation in sodium dodecylsulfate. These complement component(s) have subunit molecular weights of approximately 80 000, 45 000 and 30 000, as judged by their electrophoretic mobility in sodium dodecylsulfate.

The fact that parachloromercuribenzoic acid treatment of membranes produces no change in the two-dimensional pattern excludes disulfide bonding between membrane and complement proteins. Also, others have demonstrated that direct disulfide bonding between membrane proteins and complement proteins is not necessary for complement lysis of intact cells [21]. The complexes we observe must therefore be held together by non-covalent bonds, which are not dissociated by sodium dodecylsulfate alone. Experiments on other systems also show that sodium dodecylsulfate does not invariably dissociate non-covalent bonds in proteins [25–29]. In the present case, the complement protein complexes are clearly stabilized by disulfide bonds, and can be dissociated by sodium dodecylsulfate only after disulfide cleavage. Conceivably, disulfide bonds in complement components are crucial for their binding to membrane proteins and perhaps also with membrane lipids.

Turning to the analysis of membrane "receptors" for complement, one could argue that the attachment of complement proteins to membrane proteins occurs after cell lysis. With C6-deficient sera, however, one avoids cell lysis. The complement proteins of these sera can therefore bind only to the membrane exterior. However, we still see attachment of complement to membrane peptides 2.3, 3, 4 and 5. Proteases do not digest these peptides of intact sheep erythrocytes [30], indicating that they are not exposed or reactive at the external membrane face. Close association between these peptides and complement components therefore suggests penetration of these complement components into the target membrane as in the model in [31]. Our concept differs from Mayer's doughnut theory [10], because it deals with earlier reacting components, i.e. before C6, and may conceivably relate to C3 and/or C5.

A further interesting finding is the abnormal electrophoretic behaviour of certain peptides with dithiothreitol treatment. This is the case with albumin and an early-reacting complement component of apparent molecular weight 180 000. These peptides appear above the diagonal in two-dimensional runs. The effect is characteristic of certain globular proteins which possess one or more disulfide bonds within a peptide chain. Cleavage of these bonds causes total unfolding in sodium dodecylsulfate and results in a slight decrease in electrophoretic mobility (Kickhöfen, B., personal communication). The earlier complement component which shows this electrophoretic behaviour therefore should possess one or more such disulfide bonds.

The experimental approach we utilize offers the advantage that complement proteins are present in concentrated form on target membranes and can thus be readily subjected to analyses. It is hoped that future work along these lines will stimulate progress in molecular analyses of membrane-bound complement and finally lead to a better understanding of the attachment and lytic mechanism of complement on target membranes.

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