ISOLATION AND CHARACTERIZATION OF A C3b RECEPTOR-LIKE MOLECULE FROM MEMBRANES OF A HUMAN B LYMPHOBLASTOID CELL LINE (RAJI)

Monique BAREL, Christiane CHARRIAUT and Raymond FRADE*

Laboratoire de Biochimie des Antigènes de Membranes, ICIG, INSERM U 50 et Association Claude Bernard, 94804 Villejuif Cedex, France

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1. Introduction

Specific membrane receptors for breakdown products of the third component of human complement, C3, are expressed in several mammalian cells. At present, 5 receptors are described, i.e., C3a, C3b, C3d, iC3b and H altered C3b receptors [1]. Biochemical and molecular analysis of 2 of these, namely C3b and C3d receptors, have been approached.

Kinetics studies have shown that reversible C3b binding on a human B lymphoblastoid cell, Raji, depends on the soluble or particle-bound state of human C3b, each interaction being characterized by a different affinity constant, i.e., 10^6 M^{-1} for soluble C3b [2] or 10^7 M^{-1} for particle-bound C3b [3]. A similar affinity constant of 1.5×10^7 M^{-1} was also found for reversible particle-bound C3b binding to neutrophils [4].

Molecular analysis of C3b receptors showed that C3b receptor activity extracted from human erythrocytes membranes was carried by a 205 000 M_r glycoprotein [5], a 195 000 M_r glycoprotein [6] or 10^6, 80 000 and 60 000 M_r glycoproteins [7]. The C3b receptor activity extracted from rabbit alveolar macrophages was carried by a 62 000 M_r glycoprotein [8]. From the human B lymphoblastoid cell line, Raji, a C3d receptor activity was purified from culture medium associated with a 72 000 M_r glycoprotein [9], whereas attempts to purify C3b receptors from its membranes have been unsuccessful [10].

This is the first report of the solubilization from the membrane of the human B lymphoblastoid cell line, Raji, of a 140 000 M_r glycoprotein carrying a C3b binding activity. Some properties of this membrane component were analyzed.

Abbreviations: C3, native third component of complement; C3b, large fragment of trypsin-cleaved C3; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SDG, sucrose density gradient; BSA, bovine serum albumin; BBSS, 0.15 M NaCl 3×10^{-3} M barbital buffer (pH 7.4); VB, 3×10^{-3} M barbital buffer (pH 7.4); NP-40; Nonidet P-40 detergent; M_r, relative molecular mass; 2-ME, 2-mercaptoethanol

* To whom correspondence should be addressed

2. Materials and methods

2.1. *Cells*

Raji, a C3b receptor-positive B cell line, derived from a Burkitt's lymphoma, and CEM, a C3b receptor-negative T lymphoblastoid cell line, derived from a T ALL (acute leukocyte lymphoblastoma) were grown in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Gibco) and with 2 mM L-glutamine, 100 units penicillin/ml and 86 μM streptomycin at 37°C in a 5% CO_2 incubator.

2.2. *Purification of human C3*

C3 was purified as in [11] to homogeneity, as assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS–PAGE) and by immunoelectrophoresis with goat anti-C3 (Miles). C3b was freshly prepared from the purified C3 by trypsin cleavage under the conditions in [12].

2.3. *Detection of C3b receptors on human lymphoblastoid cells*

C3b receptors were detected on the cell surface following the specific binding of ^{125}I-C3b [2] or unlabelled C3 by indirect immunofluorescence [13] or by a specific radioimmunoassay (submitted).

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2.4. *Radiolabelling of cell surface or membrane preparations*

Cell surface was labelled with ^{125}I (carrier-free) from Amersham, by the lactoperoxidase procedure [14]. Free unbound iodine was removed by extensive washes with 0.15 M NaCl in 3×10^{-3} M barbital buffer (BBSS) (pH 7.4). Phosphorylation of Raji cells 'in vivo' or phosphorylation of Raji cell membranes 'in vitro' were performed with $^{32}P_i$ and [γ-^{32}P]ATP, respectively, according to [15]. Labelling of carbohydrate moiety of Raji membrane components was done as in [16].

2.5. *Preparation of membrane extracts*

Radiolabelled human lymphoblastoid cells (5×10^7) were lyzed by addition of 3×10^{-4} M barbital buffer (pH 7.4) at 4°C and membranes were collected by centrifugation at 100 000 × *g* for 30 min at 4°C. Lymphoblastoid cell membranes were washed 3 times in the same buffer then solubilized in 6 ml Nonidet P-40 (NP-40, Fluka) in the same buffer at 4°C for 45 min. Insoluble material was removed by centrifugation for 30 min at 100 000 × *g* at 4°C. The protein content of the solubilized membranes was estimated by the Bradford assay [17].

2.6. *Preparation of affinity chromatography beads*

10 mg of purified C3b, IgG, BSA or glycine were coupled to 2 ml cyanogen bromide-activated Sepharose 4B (Pharmacia) as in [18].

2.7. *SDS polyacrylamide gel electrophoresis (SDS–PAGE)*

SDS–PAGE (0.1% SDS, 7.5% polyacrylamide containing 0.4% bisacrylamide) was performed using the discontinuous buffer system in [19]. ^{125}I- and $^{32}P_i$-labelled proteins and [^{14}C]glucosamine-labelled proteins were detected by autoradiography and fluorography, respectively, using 3M films (Trimax XD).

3. Results and discussion

Raji cell surfaces (5×10^7 cells) were labelled with ^{125}I using lactoperoxidase method [14], then crude membranes were prepared (section 2), incubated 30 min at 4°C in 3×10^{-3} M barbital buffer (pH 7.4) (VB) containing 0.5% NP-40 and solubilized membrane components were recovered in the fluid phase after a 30 min centrifugation at 100 000 × *g*.

Radiolabelled crude membrane extract (6 ml) was incubated for 1 h at 4°C with 3 ml DEAE beads (Whatman). Unbound solubilized membrane components (effluent) were removed by centrifugation and DEAE beads were washed 5 times with 0.5% NP-40 in VB. Then elution of DEAE-bound membrane components (eluate) was performed by 6 ml 0.5% NP-40 in VB containing 600 mM NaCl. The effluent and the eluate were dialyzed overnight at 4°C against BBSS (pH 7.4) containing 0.5% NP-40. Both fractions were incubated, for 30 min at 37°C and 10 min at 4°C, with 500 μl of the following affinity chromatography beads: C3b–Sepharose, glycine–Sepharose, IgG–Sepharose or BSA–Sepharose. These beads were then washed 3 times with 4 ml 0.5% NP-40 in BBSS (pH 7.4).

When the DEAE eluate was incubated with each type of affinity chromatography bead described above, a membrane component labelled by ^{125}I, with a 140 000 M_r was eluted only by a 10^{-6} M unlabelled C3b (not by unlabelled BSA, IgG or glycine at the same concentration) from C3b–Sepharose (fig.1,A_1–A_2) but neither from glycine–Sepharose, IgG–Sepharose nor BSA–Sepharose. When DEAE effluent was treated in the same conditions, no compound was eluted from any type of affinity chromatography bead in presence of 10^{-6} M unlabelled C3b (fig.1,A_3). Attempts to use the immunoprecipitation system, i.e., soluble C3b then rabbit anti-C3, instead of affinity chromatography beads were unsuccessful in detecting this 140 000 M_r glycoprotein. Such a discrepancy is most likely related to the different affinity constants of soluble C3b or particle-bound C3b for C3b receptors on cell surface [2,3].

When the same experiments were conducted using solubilized membranes from a C3b receptor-negative cell line, CEM, no specific C3b-binding protein could be shown by SDS–PAGE analysis.

The M_r of this C3b-binding molecule was unchanged by SDS–PAGE analysis performed in the presence of a reducing agent such as 2-ME (fig.1,A_4), ruling out the presence of disulfide-linked subunits. The presence of a carbohydrate moiety on this C3b-binding molecule was supported on the one hand by its radiolabelling after intracellular labelling with [^{14}C]glucosamine (fig.1,B_1) and on the other hand by its properties of binding to *Lens culinaris*–Sepharose beads and to be eluted from these lectins by 4% *N*-acetylmannoside and *N*-acetylglucosamine (fig.1,B_2). 'In vivo' labelling of Raji cells by $^{32}P_i$ and 'in vitro' labelling of

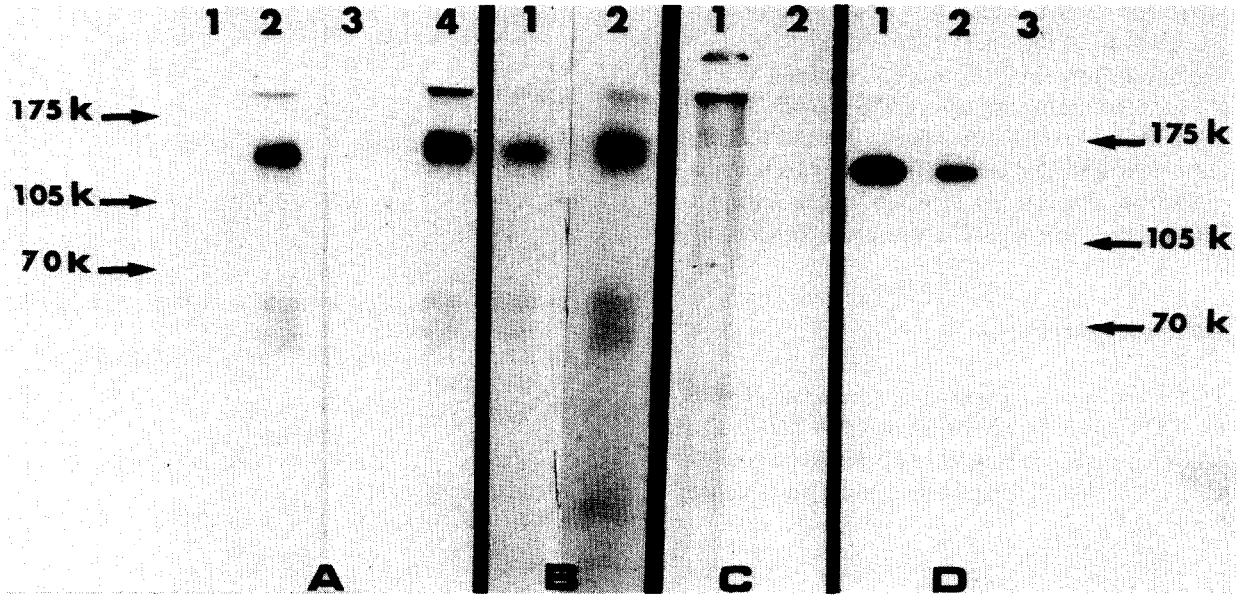

Fig.1. Analysis by SDS–PAGE of the isolated C3b-binding protein from Raji membranes: 5×10^7 cells were labelled by ^{125}I, [^{14}C]glucosamine or $^{32}P_i$, then crude membranes were prepared; membrane components were solubilized by 0.5% NP-40; radiolabelled crude membrane extracts were incubated with DEAE beads, to obtain a DEAE eluate and a DEAE effluent as in section 3. (A) ^{125}I-DEAE eluate was incubated with glycine–Sepharose, BSA–Sepharose, IgG–Sepharose beads (A1) or with C3b–Sepharose beads (A2). ^{125}I-DEAE effluent was incubated with C3b–Sepharose (A3). Then in all experiments, elution from affinity chromatography beads was performed by 10^{-6} M unlabelled C3b and analysis were realized in the absence of 2-ME. The same experiment as (A2) but analyzed in the presence of 2-ME is shown in (A4). (B) In (B1) DEAE eluate from [^{14}C]glucosamine-labelled cells, proceeded on C3b–Sepharose beads and analyzed as in (A4). In (B2) ^{125}I-DEAE eluate was incubated with *Lens culinaris*–Sepharose beads and eluted by 4% *N*-acetylglucosamine and α-methyl-D-mannoside. After dialysis, eluted components were incubated with C3b–Sepharose beads and analyzed as (A4). (C) ^{32}P-DEAE eluate from 'in vivo' $^{32}P_i$-labelled Raji cells (C1) or from 'in vitro' [γ-^{32}P]ATP labelled Raji membranes (C2), proceeded on C3b–Sepharose beads and analyzed as (A4). (D) In (D1) ^{125}I-DEAE eluate was incubated with C3b–Sepharose then elution was performed by 0.5 M acetic acid, immediately neutralized to pH 7.4. This acid-eluated C3b-binding protein was incubated with new C3b–Sepharose beads (D2) or glycine–Sepharose (D3) and elution was performed by 10^{-6} M unlabelled C3b. Analyses were realized as (A4). All samples were run on a 10% polyacrylamide gel except for (D) which were run on a 7.5% polyacrylamide gel.

Raji membranes by [γ-^{32}P]ATP before solubilization of membrane components by 0.5% NP-40, did not show any phosphorylation of the 140 000 M_r C3b-binding molecule (fig.1,C_1–C_2).

The ligand binding activity of this membrane component was tested taking advantage of its elution from C3b–Sepharose beads by 0.5 M acetic acid followed by immediate neutralization to pH 7.4. As shown in fig.1 (D_1), this acid-eluted C3b-binding protein was able to rebind to new C3b–Sepharose beads and could be eluted from this second affinity material by 10^{-6} M unlabelled C3b (fig.1,D_2–D_3). However, it should be noticed that the yield of C3b-binding protein recovered after this second elution is ~30% and this yield decreased very rapidly during prolonged incubation in 0.5 M acetic acid, suggesting that partial denaturation occurred during the obligatory acid elution procedure.

The ligand binding activity of the 140 000 M_r membrane glycoprotein has to be related to our recent study by SDG analysis of a 12 S complex formed between ^{125}I-C3b and unlabelled solubilized components from Raji membrane [20]. It was found at the expected position of a dimer formed by one molecule of the C3b (175 000 M_r) and one molecule of the 140 000 M_r membrane glycoprotein.

Moreover, we have shown by SDG and SDS analysis that C3b bound to Raji cell surfaces or incubated with solubilized Raji membrane was not cleaved either into C3bi or C3c and C3d forms [20], ruling out the presence of a detectable C3d site on the C3b molecule involved in these interactions. These data

suggest strongly that the 140 000 M_r glycoprotein extracted from Raji membrane and carrying a C3b binding affinity interacts as a C3b receptor and not as a C3d receptor. Experiments are in progress to analyze the possible relationship between this 140 000 M_r glycoprotein extracted from Raji membranes and the 72 000 M_r glycoprotein detected in Raji culture medium and carrying a C3d receptor-like activity [9].

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References

[1] Ross, G. D. (1980) J. Immunol. Methods 37, 197–211.
[2] Frade, R. and Strominger, J. (1980) J. Immunol. 125, 1332–1339.
[3] Frade, R. and Strominger, J. (1981) Immunol. Lett. 3, 173–178.
[4] Berger, M., Gaither, T. A., Hammer, C. H. and Frank, M. M. (1981) Fed. Proc. FASEB 40, abst. 4461.
[5] Fearon, D. T. (1980) J. Exp. Med. 152, 20–30.
[6] Dobson, N. J., Lambris, J. D. and Ross, G. D. (1981) J. Immunol. 126, 693–698.
[7] Gerdes, J. and Stein, H. (1980) Immunology 41, 929–936.
[8] Schneider, R. J., Kulczycki, A. jr, Law, S. K. and Atkinson, J. P. (1981) Nature 290, 789–792.
[9] Lambris, J. D., Dobson, N. J. and Ross, G. D. (1981) Proc. Natl. Acad. Sci. USA 78, 1828–1832.
[10] Dierich, M. P. and Reisfeld, R. A. (1975) J. Immunol. 114, 1676–1682.
[11] Tack, B. F. and Prahl, J. W. (1976) Biochemistry 15, 4513–4520.
[12] Bokish, V. A., Müller-Eberhard, H. J. and Cochrane, C. C. (1969) J. Exp. Med. 129, 1109–1130.
[13] Barel, M., Charriaut, C. and Frade, R. (1981) J. Immunol. Methods in press.
[14] Marchalonis, J. J., Cone, R. E. and Santer, V. (1971) Biochem. J. 124, 921–927.
[15] Pober, J. S., Guild, B. C. and Strominger, J. (1978) Proc. Natl. Acad. Sci. USA 75, 6002–6006.
[16] Kulczycki, A. jr, Krause, V., Chew Killion, C. and Arkinson, J. P. (1980) J. Immunol. 124, 2772–2779.
[17] Bradford, M. M. (1976) Anal. Biochem. 72, 248–254.
[18] Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059–3065.
[19] Laemmli, U. K. (1970) Nature 227, 680–685.
[20] Charriaut, C., Barel, M. and Frade, R. (1982) Eur. J. Immunol. in press.