

PROPERDIN- AND NEPHRITIC FACTOR-DEPENDENT
C3 CONVERTASES: REQUIREMENT OF NATIVE C3
FOR ENZYME FORMATION AND THE FUNCTION OF BOUND
C3b AS PROPERDIN RECEPTOR*·‡

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The purpose of this paper is to demonstrate the functional similarity of activated properdin (P)¹ (1) and nephritic factor (NF) (2, 3) in the generation of C3 convertase activity and to distinguish between these two related enzymes and the subsequently formed C3b-dependent C3 convertase (4) of the alternative pathway. Specifically, it will be shown that (a) the P- and NF-dependent enzymes require native C3 and not C3b as a cofactor, in addition to proactivator (factor B) and proactivator convertase (factor D̄); (b) the enzymes can efficiently turnover C3 in absence of formation of the C3b-dependent convertase; and (c) bound C3b can function as a receptor for P and thus possibly as a focus for the assembly of P-C3 convertase. In the following, the differential requirements of the formation of these three enzymes will be delineated.

Two different mechanisms of action have previously been proposed for NF. Reports from this laboratory (3) implicated properdin (P) as a cofactor in the formation of the NF-dependent C3 convertase, in addition to proactivator and proactivator convertase. Reports from Williams et al. (5, 6) indicated that NF acted via the C3b-dependent feedback without involving P. This discrepancy in results led us to reinvestigate the problem. Our present findings indicate that neither P nor the C3b-dependent feedback are essential for NF action.

Materials and Methods

Purified Components. Highly purified C3 (7), PA (8), C3PAse (1), P̄ (1), cobra venom factor (9), C4 (10), and C5 (7) were isolated as described elsewhere. C3b was prepared by either incubation of

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¹ *Abbreviations and synonyms used in this paper:* C3PA, PA, B, C3 proactivator; proactivator, factor B; C3PAse, PAse, D̄: C3PA convertase, factor D̄; NF: nephritic factor (C3NeF); NHS: normal human serum; NHS s C4,P: NHS immunochemically depleted of C4 and P; P: properdin; P̄: activated properdin.

purified C3 with EAC4^{oxy}2 (11) or incubation of purified C3 with trypsin as described elsewhere (12). C4b was prepared from isolated C4 by treatment with trypsin (1%, wt/wt) for 2 min at 22°C.

Partially Purified NF. NF was isolated repeatedly from the serum of two patients with hypocomplementemic glomerular nephritis using the method previously described (3) with the following modifications. After TEAE-cellulose chromatography the breakthrough was concentrated, dialyzed against 0.02 M sodium phosphate buffer, pH 6.0, and applied to a column of CM-Sephadex C-50 equilibrated with the same buffer. After washing, NF was eluted and separated from P with a linear NaCl concentration gradient. NF eluted in fractions having a conductivity of 11–14 mmho/cm, while P was found in the 21–24 mmho/cm eluate. All partially purified NF preparations used were free of P. By Ouchterlony analysis, immunoelectrophoresis, and polyacrylamide gel electrophoresis, only IgG could be detected as a contaminant. This protein was not removed because NF has previously been shown to be distinct from immunoglobulin (3).

Preparation of Immune Adsorbents. Anti-C4 and anti-P-immune adsorbents were prepared by coupling the IgG fractions of the respective monospecific antiserum to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc. Piscataway, N. J.) using the method of March et al. (13).

Immune Adsorption of Normal Human Serum (NHS). Fresh NHS containing 0.01 M EDTA was applied to the appropriate immune adsorbent column at a flow rate of 10 ml/h at 4°C. The adsorbed sera were concentrated to their original volumes and dialyzed against barbital buffer, pH 7.4, containing either 2×10^{-3} M EDTA or 1×10^{-3} M MgCl₂. Serum depleted of C4 or P or both C4 and P, was tested for the presence of the respective components by Ouchterlony analysis and hemolytic assays. In all cases, immune adsorption was essentially complete.

Polyacrylamide Gel Electrophoresis. Electrophoresis in 6% polyacrylamide gels at pH 8.6 was performed according to published methods (14). Gels to be eluted were first subjected to electrophoresis for 4 h at 4°C with 50 µg of carboxymethylated human serum albumin. After replacing the buffer in the electrode chambers, 100-µl samples of NF were applied to the gels and electrophoresis continued for an additional 3 h. Gels were sliced into 2-mm segments which were eluted for 12–18 h at 4°C with 0.2 ml isotonic barbital buffer, pH 7.4.

Assay for NF Activity. NF activity was assessed by its effect on C3 in normal serum or on isolated C3 in presence of appropriate cofactors as determined by either immunoelectrophoresis or effective molecule titration (15). One NF unit is defined as the amount of NF required to consume 50% of 15 µg of C3 within 30 min at 37°C in a total vol of 20 µl containing the isolated cofactors.

Preparation of EAC4b,3b. EAC4b,3b was prepared by incubating EAC1,4b with nonoxidized C2 for 8 min at 30°C (11). The resulting EAC1,4b,2a cells were then converted to EAC4b,3b by treatment of 10^9 cells with 95 µg purified C3 and subsequent incubation for 120 min at 37°C in 0.01 M EDTA to remove C1 and C2a.

Hemagglutination and Hemagglutination Inhibition. This was performed in microtiter U plates (Cooke Laboratory Products Div., Dynatech Laboratories Inc., Alexandria, Va.). Agglutination of EAC4b,3b by isolated, \bar{P} was carried out with 25 µl of 10^8 cells/ml and 25 µl of \bar{P} ranging from 1–1,000 ng/sample. As a control, EAC4b was used. Agglutination inhibition utilized various proteins in amounts of 125–10,000 ng/sample. 25 µl containing 6 ng of \bar{P} and 25 µl of EAC4b,3b were added to each sample. The plates were incubated with agitation for 30 min at 37°C and kept overnight at 4°C. The degree of agglutination was read by inspection and graded from 1+ to 4+.

Results

Lack of Requirement of P in the Formation of NF-C3 Convertase. The P dependence of NF-C3 convertase was reinvestigated in the following manner: NF was incubated at 37°C for 30 min with (a) NHS, (b) NHS immunochemically depleted of C4 (NHS \bar{s} C4), (c) NHS immunochemically depleted of P (NHS \bar{s} P), and (d) NHS immunochemically depleted of both C4 and P (NHS \bar{s} C4,P). Subsequently, C3 consumption was determined and found to be virtually identical in all four sera. Fig. 1 shows the dose response of NF in NHS \bar{s} C4 and NHS \bar{s} C4,P. In order to assure that NF activity represented a single entity free of \bar{P} , the activity distribution of NF was examined after polyacrylamide gel electrophoresis. Under the conditions employed, \bar{P} has been shown not to enter the

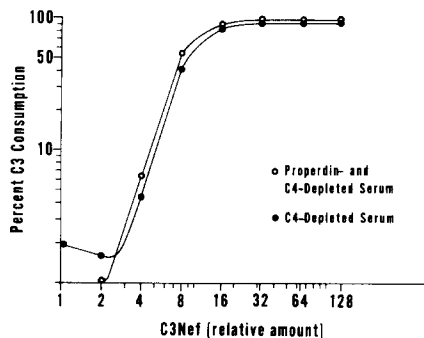


FIG. 1. Consumption of C3 by NF in serum depleted of C4 or C4 and P 10 μ l of NF and 10 μ l of the above reagents were incubated for 30 min at 37°C. The reaction was stopped by addition of 1 ml of ice-cold 0.04 M EDTA solution. Residual C3 activity was quantitated by effective molecule titration.

gel but to migrate toward the cathode (1). The activity detected by the NHS \bar{s} C4,P was eluted from segments 5 and 6 of the gel which was sliced into 32 segments. This position corresponds to that of the slowly migrating portion of IgG, which is considerably different than that of \bar{P} .

Cofactor Requirements of the Formation of NF- and \bar{P} -C3 Convertases. Since no requirement of P could be demonstrated in the previous experiments, a qualitative comparison was performed of the necessary cofactors for the action of NF and \bar{P} on C3. NF or \bar{P} were mixed with C3, PA, and PAse in the presence of 6×10^{-4} M MgCl₂. After 30 min incubation at 37°C, the amount of C3 consumption was determined by hemolytic titration. The experimental conditions and results of one such experiment are listed in Table I. They indicate that NF and \bar{P} are functionally similar because they share the requirement for C3, PA, PAse, and magnesium ions.

C3 Consumption by NF- and \bar{P} -C3 Convertases in Absence of C3b-Feedback Amplification. Dose response experiments indicated a widely differing requirement of PAse for the formation of the various C3 convertases. For 50% C3 consumption the C3b-dependent enzyme needed approximately 17 times more PAse than the NF- or \bar{P} -C3 convertases. The cobra venom factor-induced enzyme needed approximately 10 times more PAse than the latter two enzymes. As shown in Fig. 2, it is possible therefore to obtain full expression of activity of NF- or \bar{P} -C3 convertases under conditions which do not allow formation of the C3b-dependent enzyme. These results directly demonstrate the efficiency of NF- and \bar{P} -C3 convertases to turnover C3 in the absence of the C3b feedback.

Dose response experiments for magnesium ions were performed with NHS \bar{s} C4,P. To achieve 50% C3 consumption by either NF- or \bar{P} -C3 convertase, 2.3×10^{-4} M Mg⁺⁺ was needed. This value is twice that characteristic for the cobra venom factor or C3b-dependent enzymes. This observation and results presented below indicate that metal ions act at two different steps of the alternative pathway.

Requirement of Native C3 and not of C3b. The question arose as to whether the \bar{P} - or NF-C3 convertases utilize C3 or C3b. In order to obtain an answer it was necessary to find out whether the processes of formation of the enzymes and

TABLE I
Cofactor Requirements for NF- and \bar{P} -C3 Convertases*

Cofactor	C3 consumption by:		
	NF	\bar{P}	Buffer
	%	%	%
C3 + PA + PAse + Mg ⁺⁺	98.8	98.8	2.1
C3 + PA + PAse + EDTA	0.0	0.0	0.0
C3 + Mg ⁺⁺	3.9	ND‡	3.4
C3 + PA + Mg ⁺⁺	1.8	0.0	0.0
C3 + PAse + Mg ⁺⁺	0.0	0.0	6.7

* Reaction mixtures contained various combinations of 9.5 μ g, C3, 2.0 μ g PA, 6 ng PAse, 6×10^{-4} M MgCl₂, 10^{-2} M EDTA, 140 ng \bar{P} , and 2 units NF which were incubated at 37°C for 30 min. Reactions were stopped by addition of EDTA (final concentration, 0.01 M) and lowering the temperature to 4°C. C3 consumption was determined by effective molecule titration.

‡ ND, not done.

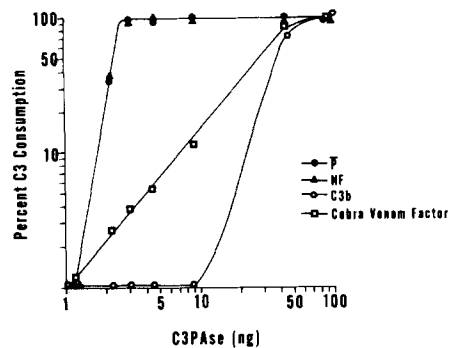


FIG. 2. Differential requirement of proactivator convertase (\bar{D}) for the formation of various C3 convertases. Reaction mixtures were prepared as described in the legend to Table I with the indicated amounts of PAse. The various enzymes were generated with, respectively, 140 ng of \bar{P} , 2 units NF, 1–4.5 μ g C3b, or 0.3–0.9 μ g cobra venom factor.

their action on substrate can be dissected. It was found that whereas formation of both enzymes required magnesium ions, as emphasized above, action of the formed enzymes on substrate proceeded in presence of EDTA. Utilizing the two-step reaction it was possible to demonstrate, as shown in Table II, that native C3 rather than C3b served as essential cofactor in the formation of the two enzymes. If C3 was omitted in the first step of the reaction, no enzyme activity was demonstrable in the second step in which excess C3 was added as substrate. When C3b was present in 24-fold excess over C3 during the first phase, a 54–56% reduction in C3 consumption was observed during the second phase. Whether C3b inhibits the formation of the enzyme or the action of the formed enzyme cannot be distinguished on the basis of these experiments.

Direct Interaction of \bar{P} with Native C3 and with C3b. Addition of isolated \bar{P} to EAC4b,3b resulted in strong agglutination of the cells. Agglutination was not

TABLE II
*C3 and not C3b Requirement for Formation of NF- and \bar{P} -C3
 Convertases**

Reaction mixture plus:		C3 consumption by:		
C3	C3b	NF	\bar{P}	Buffer
μg	μg	%	%	%
0.0	9.5	0.0	0.0	0.0
9.5	0.0	61.6	76.0	0.0
1.9	0.0	88.6	57.3	0.0
1.5	37.5	43.4	22.2	3.8
0.95	0.0	90.9	45.2	8.6
0.48	0.0	96.3	45.6	5.1
0.24	0.0	95.7	46.4	1.9
0.19	0.0	97.1	35.3	0.0

* Reaction mixtures containing 2.0 μg PA, 7.5 ng PAse, and 10^{-3} M MgCl_2 were incubated 15 min at 37°C with either 2 units NF, 70 ng \bar{P} , or buffer in the presence of various amounts of C3 and/or C3b. After addition of EDTA (final concentration, 0.01 M), 9.5 μg of C3 (in 0.02 M EDTA) was added and incubation allowed to proceed for an additional 30 min at 37°C. C3 consumption was determined by effective molecule titration.

‡ The C3b used was active in forming the C3b-C3 convertase in presence of sufficient amounts of PAse and B as shown in Fig. 2.

observed when \bar{P} was added to EAC4b. These results indicate that \bar{P} is capable of binding to C3b and not to C4b and that it possesses more than one binding site for C3b. Approximately 250 \bar{P} molecules/cell were sufficient to produce clearly detectable agglutination (2 ng \bar{P} /2.5 $\times 10^6$ cells). This reaction of \bar{P} with C3b afforded to determine whether native C3 competes with C3b for \bar{P} sites. As shown in Table III, native C3 in solution totally abrogated the agglutination of EAC4b,3b. A similar inhibition was observed with soluble C3b, but not with either C4, C4b, C5, or proactivator.

NF was tested in the agglutination reaction in amounts ranging from 0.25 to 16 units. No agglutination was observed. On a functional basis, one unit of NF corresponds to approximately 70 ng of \bar{P} . The inability of NF to agglutinate C3b-bearing cells may be a reflection of an affinity for C3b which is lower than that of \bar{P} .

Kinetics of Formation and Decay of NF- and \bar{P} -C3 Convertases. Fig. 3 illustrates rapid formation of the enzymes at 37°C in presence of magnesium ions with maximal activity being reached between 8 and 12.5 min. The decay rates were estimated in presence of 1×10^{-3} M Mg^{++} . To determine the decay rate of NF-C3 convertase in 0.01 M EDTA, the chelator was added at 10 min at 37°C after formation of the enzyme. Estimated from the slopes of the curves in Fig. 3, the decay rates for \bar{P} - and NF-C3 convertases were respectively, 1.9%/min and 1.4%/min. In presence of EDTA the rate was 2.9%/min for NF-C3 convertase.

Enzymatically Active C3 Proactivator in Absence of Fragmentation of the

TABLE III
Interaction of \bar{P} with C3 and C3b Demonstrated by Agglutination and Agglutination Inhibition*

Amount of soluble protein ng	Degree of agglutination in presence of:					
	C3	C3b	C4	C4b	C5	PA
0	4+	4+	4+	4+	4+	4+
125	4+	4+	4+	4+	4+	4+
250	4+	4+	4+	4+	4+	4+
500	1+	1+	4+	4+	4+	4+
1,000	0	0	4+	4+	4+	4+
2,000	0	0	4+	4+	4+	4+
4,000	0	0	4+	4+	4+	4+
8,000	0	0	4+	4+	4+	ND‡

* EAC4b,3b: 25 μ l of 10^8 cells/ml; \bar{P} : 6 ng; and buffer or protein solution: 25 μ l containing protein as indicated; 30 min, 37°C with agitation, then held at 4°C overnight.

‡ ND, not done.

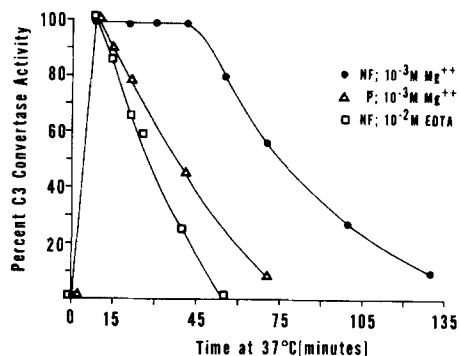


FIG. 3. Formation and decay of \bar{P} - and NF-C3 convertases as a function of time at 37°C. The Mg^{++} concentration was 10^{-3} M. In one experiment, EDTA was added to NF-C3 convertase 10 min after initiation of enzyme formation. At the indicated time intervals, 20 μ l of the enzymes were sampled into 10 μ l of EDTA solution containing 9.5 μ g C3. C3 consumption was determined after 30 min at 37°C by effective molecule titration.

Molecule. Examples of proactivator activation have been described where cleavage of the molecule was apparently not a prerequisite (16). When PA which had participated in turnover of C3 by NF- or \bar{P} -C3 convertase (formed with low PAse concentration) was examined by immunoelectrophoresis, the pattern was indistinguishable from that of native PA as shown in Fig. 4. In contrast, formation of the C3b-dependent enzyme (at high PAse concentration) was associated with typical and complete fragmentation of PA. Irrespective of PA cleavage, as detected by immunoelectrophoresis, the C3 present in the reaction mixtures was totally consumed except in the controls, which never exceeded 6% C3 consumption. This was determined by immunochemical analysis and by effective molecule titration. The reaction mixtures contained 9 μ g PA, 57 μ g C3,

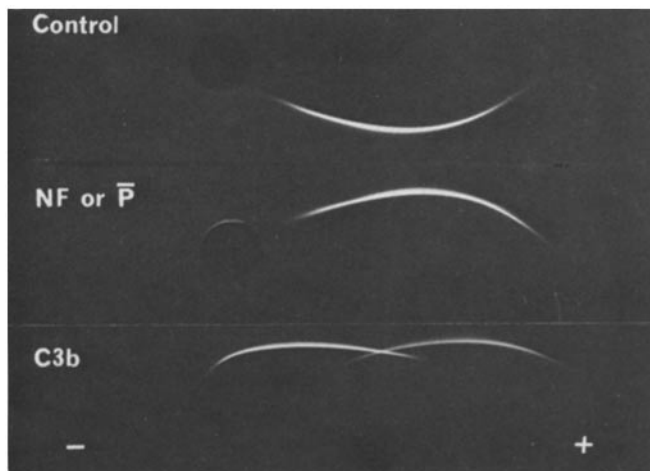


FIG. 4. Utilization of proactivator by NF of \bar{P} without apparent fragmentation of the molecule, as detected by immunoelectrophoresis. The top panel is representative of (a) isolated, untreated PA and (b) PA treated with the required cofactors with the exception of either NF, \bar{P} , C3, PAse, or Mg^{++} . The middle panel is representative of PA treated at low PAse concentrations with all cofactors including either NF or \bar{P} . The immunoelectrophoretic analysis was performed after the enzymes had converted all of 57 μg of C3. The bottom panel shows for comparison complete cleavage of PA after treatment with C3b at high PAse concentration. The figure is a composite of two separate experiments. Electrophoresis was carried out in 1% agarose in barbital buffer, pH 8.6, containing 0.01 M EDTA. The patterns were developed with goat antiserum to human PA.

18 ng PAse, 10^{-3} M Mg^{++} , 8 or 16 units NF, or 0.5 or 1 μg \bar{P} , in a total vol of 50 μl . For the demonstration of the C3b-dependent enzyme, the amount of PAse was increased to 80 ng and 5 μg C3b was added instead of NF or \bar{P} . The results are compatible with the concept that the C3-cleaving activity may be expressed by the proactivator without the typical proteolytic fragmentation.

Apparent Immunochemical Unrelatedness of NF and P. The observed functional similarity of NF and \bar{P} prompted an analysis as to whether the proteins share antigenic determinants. 200 μl of a solution containing 297 U of NF and 2 μCi of [^{125}I]IgG were applied to a P-immune adsorbent column which was prepared with antiserum made to \bar{P} isolated from fresh serum. This column had been shown to remove 100% of P from 50 ml of NHS (approximately 1,000 μg). Compared to the total applied material, the unadsorbed material contained: 74% of NF activity, 67% of the radioactivity, and 58% of the protein. The 1 M acetic acid-1 M NaCl eluate contained no detectable NF activity, and only traces of radioactivity and protein. The recovery of NF activity relative to that of radioactivity and protein indicates that a preferential adsorption of NF did not occur and that therefore NF was not detected by the antiserum to \bar{P} used. This result was confirmed in an experiment in which the same immune adsorbent column was used to remove P from 1 ml of NF-containing serum from a patient with nephritis. While P was not detectable in the adsorbed serum, NF activity was undiminished (Fig. 5). The detection limit for P was 6.25% of the original P concentration. The same antiserum failed to yield a precipitin line

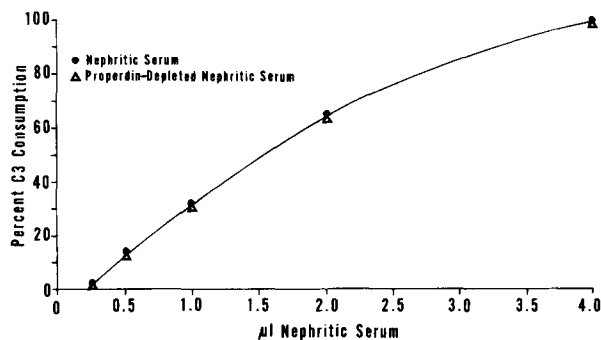


FIG. 5. Complete recovery of NF activity after removal of P from nephritic serum using immune adsorption. Quantitative relationship of C3 consumption in normal serum by NF-containing serum before and after removal of P. The reaction mixtures of 50 μ l contained: 20 μ l NHS, the indicated volumes of nephritic serum, and Mg^{++} at a final concentration of 10^{-3} M.

with NF and NF failed to inhibit the formation of a \bar{P} -anti- \bar{P} precipitin line in double-diffusion-in-gel experiments. NF-containing serum adsorbed with an NF-immune adsorbent lost all NF while retaining P in undiminished amounts.

Discussion

It has become apparent that NF and \bar{P} are functional equivalents. In the fluid phase both factors require for their action on C3 the participation of native C3, proactivator (factor B), and proactivator convertase (factor \bar{D}). Both factors have similar requirements for a defined magnesium ion and proactivator convertase concentration which are different from those of C3b and cobra venom factor. Both factors give rise to the formation of C3 convertases which, after their formation, are active in the presence of EDTA. The \bar{P} -dependent C3 convertase is formed in absence of NF and the NF-dependent C3 convertase is assembled without \bar{P} . This striking similarity in function suggests a structural relationship between NF and \bar{P} . The possibility was considered, therefore, that NF is a derivative of \bar{P} . Conceivably, a specialized proteolytic enzyme might cleave \bar{P} such that its mol wt is reduced to 150,000 dalton without loss of biological activity. This hypothesis has become improbable because, in addition to the known physicochemical differences, the present work has failed to demonstrate an immunochemical relationship between NF and \bar{P} . Our findings and conclusions are at variance with those of Daha et al. (17) who recently reported that NF may be an altered form of the normal serum protein P. If, as we propose, NF and \bar{P} are immunochemically unrelated proteins, the role of the normal serum analogue of NF (3) remains to be defined.

In the first report from this laboratory on the mechanism of action of NF, it was stated that in addition to native C3, proactivator, and proactivator convertase, P was also a required factor (3). At that time the P requirement was deduced from experiments showing total lack of C3 consumption by isolated NF in P-depleted serum. P depletion was accomplished by immune adsorption in presence of EDTA followed by addition of $MgCl_2$ to overcome the chelating effect

of EDTA. Optimal levels of magnesium were determined by the ability of cobra venom factor to cause C3 consumption. From the data reported in this paper it has become apparent that the \bar{P} - or NF-dependent steps require a higher magnesium ion concentration than the cobra venom factor- or C3b-dependent steps. Hence, the previously reported lack of NF activity in P-depleted serum can now be explained by the experimental design utilized before the critical role of the magnesium ion concentration in the \bar{P} or NF reaction steps was appreciated. It is now clear that divalent cations are needed in at least two reactions of the alternative pathway, the \bar{P} - or NF-dependent and the C3b-dependent steps.

Whereas these studies have shed new light on NF, they have furthered our understanding of the physiological protein, P. In the following we would like to discuss (a) the tentative subunit structure of \bar{P} -C3 convertase, (b) the control of the enzyme by C3b, and (c) the localizing function of bound C3b in the assembly of \bar{P} -C3 convertase.

That \bar{P} can bind to C3b-bearing erythrocytes and thereby can enhance the function of PA and PAse has been reported in abstract form by Fearon and Austen (18). We have now shown that EAC4b,3b cells are agglutinated by \bar{P} and that this agglutination is inhibitable by soluble C3b. It is of considerable functional significance that comparable amounts of native, soluble C3 can inhibit this reaction to approximately the same extent. These experiments indicate that \bar{P} can enter into direct physical contact with C3 and C3b. The formation of weak, reversible complexes between P and native or altered C3 was recently also shown by Chaptis and Lepow (19). Our results also show that native C3 is needed for the generation of \bar{P} -C3 convertase and that C3b inhibits either the formation or the function of that enzyme. That C3 represents the hydrazine-sensitive factor A of the P system has been reported from this laboratory in 1972 (4) and confirmed by Goodkofsky et al. (20). In 1974 we showed that in absence of C3b, native C3 was an essential requirement for the function of the \bar{P} -dependent alternative pathway (1). We now propose that the binding of native C3 to \bar{P} is a necessary step in the assembly of \bar{P} -C3 convertase, and affords the subsequent assimilation of PA. The utilization of native C3 by \bar{P} appears biologically sound because in the initial phase of the alternative pathway C3b is apparently not available. Preliminary estimates of the molar ratio of PA to \bar{P} at maximal enzyme activity have yielded a value of 4. This would suggest that each of the four subunits of \bar{P} (21, 22) in the \bar{P} ,C3 complex binds one molecule of PA. Since cleavage of PA does not appear to be a prerequisite for expression of its enzymatic function, the essential role of PAse in this reaction is difficult to interpret. This protein may effect allosteric functions in addition to its demonstrated enzymatic action. That PA can be enlisted as a C3-cleaving enzyme without being activated by enzymatic fragmentation, has been strongly suggested earlier through observations with a pathological serum. In this serum C3 was cleaved in the cold via the alternative pathway without evidence for proactivator cleavage or consumption of proactivator activity (16).

One proposed concept of the dynamics of the alternative pathway considers the C3b-dependent positive feedback the driving force of the entire reaction, and, by implication, the \bar{P} -dependent events merely a means of supplying the first few C3b molecules (23). Our studies now show that the \bar{P} -dependent enzyme

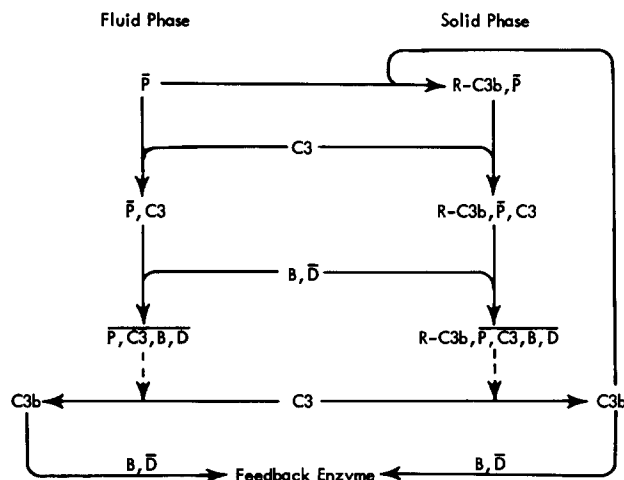


FIG. 6. Proposed mechanism for the assembly of \bar{P} -C3 convertase and for the focussing function of bound C3b. Specifically, the scheme proposes that (a) C3 and proactivator (B) are utilized in native form, (b) proactivator convertase (\bar{D}) functions in this reaction in an unknown manner, (c) bound C3b (R-C3b) serves as a receptor for \bar{P} and may thus allow the assembly of a solid phase \bar{P} -C3 convertase, and (d) soluble C3b gives rise to the formation of the feedback enzyme as well as to interference with the assembly or function of \bar{P} -C3 convertase (not indicated in the scheme). NF is envisaged to function in a manner similar to that of \bar{P} .

acts efficiently in absence of amplification by the C3b feedback. This fact could be demonstrated only after it was possible to generate \bar{P} -C3 convertase without simultaneously generating C3b-dependent C3 convertase. This was achieved by manipulating the concentration of proactivator convertase. Physiological conditions do, of course, allow the feedback to come into play.

We propose the following tentative concept for formation, structure, and function of the \bar{P} -dependent enzyme. \bar{P} binds C3 and the complex binds PA. The most probable number of molecules of PA bound by one \bar{P} -C3 complex is four. Although PAse is needed, PA is not necessarily cleaved. The multimolecular complex turns over C3, splitting it into C3a and C3b. The accumulating C3b initiates the formation of the C3b-feedback enzyme and suppresses the activity of the \bar{P} -C3 convertase. The enzyme can assemble and function in the fluid phase after activation of P by an unknown mechanism (24). It can probably also assemble on the surface of particles or cells. To date, only one mode of binding of \bar{P} to particles or cells is known: bound C3b serves as receptor for \bar{P} . Bound C3b thus fulfills a focussing function in the binding of \bar{P} and probably in the assembly of \bar{P} -C3 convertase (Fig. 6). The reported occurrence of P in tissue lesions (25-27) and on the surface of cells (R. E. Rosenfield, personal communication) may therefore be secondary not only to the activation of P but to the prior fixation of C3b. Efficient lysis of cells bearing C3b may thus involve the assembly of \bar{P} -dependent enzymes (footnote 2 and references 18, 28, and 29).

² Medicus, R. G., O. Götze, and H. J. Müller-Eberhard. Manuscript in preparation.

Work is underway which aims at the direct demonstration of the \bar{P} -containing, enzymatically active multimolecular complex in cell-free solution.

In view of the demonstrated ability of P to bind C3 and C3b, we wish to raise the possibility that P is related to the cellular immune adherence receptor. This receptor reacts with C3 and C3b, as experiments with human lymphoblastoid cells and peripheral lymphocytes have shown (29). The functional similarity of the soluble protein and the cellbound receptor suggests that the two entities have structural characteristics in common. An investigation is in progress to explore the antigenic relationship of P to the receptor.

Summary

Two complex enzymes were assembled that both converted C3 to C3b, one consisting of activated properdin (\bar{P}), native C3, proactivator (PA) and proactivator convertase (PAse), and the other of nephritic factor (NF) and the same three cofactors. By maintaining a critical concentration of PAse, the \bar{P} -C3 convertase and the NF-C3 convertase were shown to function efficiently without formation of the C3b-feedback enzyme. The former two enzymes are distinct from the C3b-dependent C3 convertase in that they utilize native C3 instead of C3b and PA in an apparently uncleaved form. The \bar{P} - and NF-C3 convertase express maximal activity within approximately 10 min at 37°C and decay with a half-life of 35 min at 37°C, which is in contradistinction to the reported lability of the C3b-feedback enzyme. \bar{P} - and NF-C3 convertases are inhibited by their product C3b, which may constitute a heretofore unknown control of the alternative pathway. A direct physical interaction of \bar{P} with native C3 and C3b was demonstrated by agglutination of C3b-bearing erythrocytes and by agglutination inhibition. Bound C3b thus constitutes the only known receptor of \bar{P} and may fulfill an important localizing function for \bar{P} and the \bar{P} -C3 convertase in vivo. Although \bar{P} and NF form functionally similar enzymes, they act independently of each other and are apparently immunochemically unrelated proteins.

Addendum. The work described above implied that NF- and \bar{P} C3 convertases are enzymes that function in parallel. On the basis of new evidence, we have come to recognize that in normal serum the NF analogue-dependent enzyme and the P-dependent enzyme assemble and act sequentially in the alternative pathway. The first enzyme appears to function as the P-receptor-forming enzyme and the second enzyme as the alternative C5 convertase.

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