AUTOCATALYTIC ACTIVATION OF THE PROENZYME FORM OF THE C1s SUBUNIT OF THE FIRST COMPONENT OF COMPLEMENT

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Summary. The autocatalytic activation of the proenzyme form of the C1s subunit of the first component of complement is reported for the first time. Incubation of the purified proenzyme at 37° and pH 7.4 results in the evolution of esterolytic activity according to a second-order autocatalytic rate law. The lag phase portion of the sigmoidal activation curve can be shortened either by increasing the proenzyme concentration or by addition of the activated C1s subunit.

The first component of human complement, C1, occurs in serum as an inactive macromolecular complex which is reversibly dissociable by treatment with EDTA into three subunits: C1q, C1r, and C1s (1). Binding of C1 to antigen-antibody complexes via C1q activates C1s, thus initiating the classical pathway of complement activation (2).

A considerable body of evidence has accumulated indicating that C1s exists as a zymogen in the native C1 complex and is activated by limited proteolysis (3-5). An autocatalytic mechanism for the activation of C1s was first proposed on the basis of kinetic studies utilizing partially purified preparations of macromolecular C1 (6). However, the results of subsequent studies utilizing purified C1 subunits appeared inconsistent with an autocatalytic model, since it was found that solutions of C1s are much more readily activated by the addition of C1r than $C1\bar{s}$, the activated form of the C1s subunit.

In an attempt to resolve this apparent discrepancy, we have reinvestigated the question of the autocatalytic activation of C1s using solutions of purified C1s and $C1\bar{s}$. The present communication is a report of the results of this study.

Methods. Both C1s and $C1\bar{s}$ were purified at 4° from a euglobulin fraction prepared from human serum (1). Details of these procedures will be published elsewhere.

$C1\bar{s}$ was assayed at 25° in the presence of 0.057M potassium phosphate, pH 7.4 containing 1 mM EDTA using the chromogenic sub-

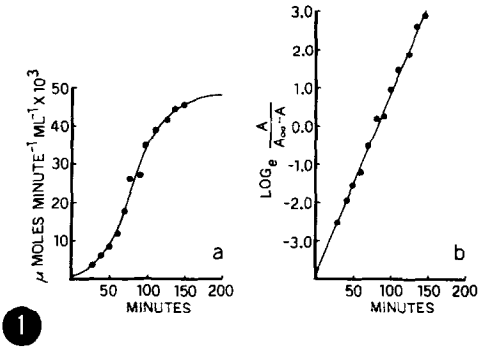
strate N-carbobenzoxy-L-tyrosine-p-nitrophenyl ester (7). The molar extinction coefficient of p-nitophenol at 402 nm and pH 7.4 was taken as 1.46×10^4 (8).

Iterative linear regression analysis was used to fit activation curves to the integrated rate law describing a second-order autocatalytic reaction:

$$\log_e A/(A_\infty - A) = kA_\infty t + \log_e A_o/(A_\infty - A_o)$$

where k is the second-order autocatalytic rate constant, A_∞ is the final activity and A_o is the activity at zero time.

Results and Discussion. The present results demonstrate that purified C1s is susceptible to autocatalytic activation by $C1\bar{s}$. The evolution of esterolytic activity in solutions of C1s at 37° and 7.4 obeys a second-order autocatalytic rate law as shown in Figure I. In accordance with an autocatalytic model, the lag phase portion of the sigmoidal activation curve can be shortened either by increasing the proenzyme concentration (Figure IIa) or by addition of the activated C1s subunit (Figure IIb). Since



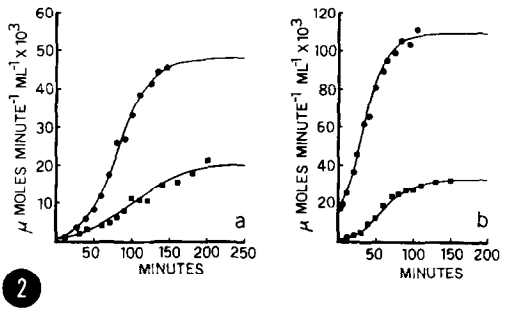


Figure Ia shows the autocatalytic activation of C1s (0.611 mg/ml) in 0.057 M potassium phosphate, pH 7.4 containing 1mM EDTA at 37°. The curve is calculated from the equation for an autocatalytic reaction where $k = 9.69 \times 10^{-4}$, $A_\infty = 48.2$ and $A_o = 1.01$.

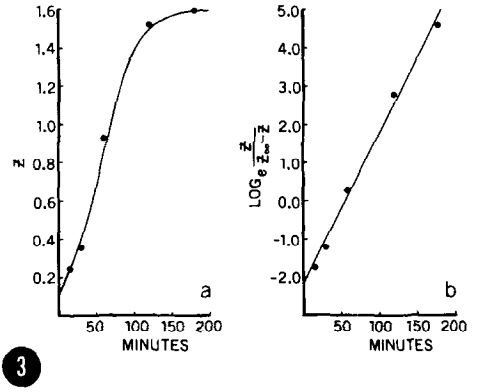
Figure Ib illustrates the agreement of the data in Figure Ia with the integrated form of the autocatalytic equation.

Figure IIa depicts the effect of zymogen concentration on the autocatalytic activation of C1s in 0.057 M potassium phosphate, pH 7.4 containing 1mM EDTA at 37°: ●—●, 0.611 mg/ml; ■—■, 0.252 mg/ml.

Figure IIb illustrates the accelerated autocatalytic activation of C1s in the presence of added $C1\bar{s}$ in 0.01 M HEPES, pH 7.4 at 37°: ■—■, 1.8 ml C1s (0.659 mg/ml) plus 0.2 ml buffer; ●—●, 1.8 ml C1s (0.659 mg/ml) plus 0.2 ml $C1\bar{s}$ (0.675 mg/ml).

the activation of C1s is a proteolytic process, we conclude that proteolytic activation of C1s can be added to the limited list of known proteolytic events mediated by $C1\bar{s}$.

Since C1r is known to accelerate the activation of C1s, it is of interest to re-examine the kinetics of activation of C1s in solutions containing C1r. Sakai and Stroud have reported an experiment in which purified C1s was treated with diisopropyl-fluorophosphate, dialyzed and then activated by addition of purified C1r (9). Plotting the number of hemolytic sites generated in the $C1\bar{s}$ assay against incubation time yielded a sigmoidal activation curve. Our analysis of this data, shown in Figure III, clearly demonstrates that the activation of C1s in the presence of C1r obeys a second-order autocatalytic rate equation.



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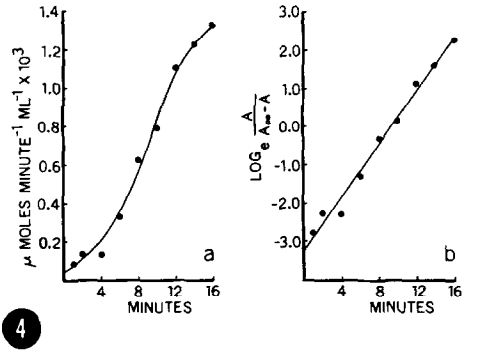

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Figure IIIa shows the autocatalytic activation of C1s in the presence of added C1r. Z is the number of hemolytic sites generated in the $C1\bar{s}$ assay. The curve is calculated from the equation for an autocatalytic reaction where $k = 2.56 \times 10^{-2}$, $A_\infty = 1.62$ and $A_o = 0.139$. The data are taken from (9).

Figure IIIb demonstrates the agreement of the data in Figure IIIa with the integrated form of the autocatalytic equation.

Figure IVa shows the autocatalytic activation of a partially purified preparation of C1. Unactivated euglobulin was incubated in 0.057 M potassium phosphate, pH 7.4 containing 1mM EDTA at 37°. Timed aliquots were diluted 4-fold with cold buffer additionally containing 0.15 M KCl and then assayed. The curve is calculated from the equation for an autocatalytic reaction where $k = 0.237$, $A_\infty = 1.46$ and $A_o = 5.53 \times 10^{-2}$.

Figure IVb demonstrates the agreement of the data in Figure IVa with the integrated form of the autocatalytic equation.

The finding that purified C1s is capable of autocatalytic activation is in accord with earlier investigations utilizing partially purified preparations of macromolecular C1 (6). In these studies it was found that adjustment of the euglobulin fraction to physiological conditions of pH, temperature and ionic strength resulted in the activation of C1s according to a second-order autocatalytic rate law. As shown in Figure IV, our observations confirm that the activation of C1 obeys autocatalytic kinetics.

Autocatalytic activation of C1s has now been demonstrated at three levels of molecular complexity: in solutions containing only C1s, in solutions containing both C1s and C1r, and in partially purified preparations of macromolecular C1. The simplest interpretation of these findings is that the subunit actually catalyzing the proteolytic activation of C1s is, in each case, the activated form of C1s itself.

The role played by C1r in the activation of C1s should be critically re-evaluated in light of these findings, since it is currently held that C1r acts proteolytically to catalyze the activation of C1s (2). Activation of C1s in the presence of C1r exhibits at least two features which appear inconsistent with the putative enzymatic function of C1r. First, stoichiometric rather than catalytic quantities of C1r are required for optimal activation of C1s (5). Secondly, progress curves for such reactions exhibit an initial phase in which the rate of activation increases with time, a feature characteristic of autocatalytic processes not exhibited by simple catalytic systems (5). By contrast, tryptic activation of C1s requires only catalytic quantities of trypsin and progress curves thus generated exhibit the kinetics of a typical catalytic process (5). We suggest therefore that C1r does not function catalytically in the activation of C1s, but rather interacts stoichiometrically with C1s and/or $C1\bar{s}$ to potentiate the inherent tendency of this zymogen-enzyme pair to undergo autocatalytic activation.

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