ENHANCEMENT OF THE HEMOLYTIC ACTIVITY OF THE SECOND COMPONENT OF HUMAN COMPLEMENT BY OXIDATION*,‡

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(Received for publication 10 July 1967)

One of the unresolved problems concerning the mechanism of immune cytolysis is the pronounced lability of the enzyme, C'4,2a, which activates the third component of complement (C'3) and which itself is derived from the second (C'2) and fourth (C'4) components (1). While the lability of this enzyme may be highly advantageous in vivo because it limits the extent of a complement reaction thereby preventing depletion of the third component, it renders exploration of the enzyme in vitro extremely cumbersome. Thus, an intermediate product of immune hemolysis which is characterized by the presence of C'4,2a on the cell surface, has been shown to undergo rapid decay (2) with concomitant dissociation of hemolytically inactive C'2 (3). The cause of the decay to date has eluded students of complement (4, 5).

The present study provides a new approach to the problem. In exploring the effect of sulfhydryl reagents on the hemolytic activity of human C'2, it was found that a greater than 10-fold enhancement of C'2 activity could be elicited by defined chemical modification of the C'2 molecule. Using modified C'2 for the generation of C'4,2a, the enzyme no longer exhibited the marked lability so characteristic of the natural compound. These observations may aid in alleviating certain technical difficulties in research on human complement; they may lead to the definition of the groups responsible for activity and stability of the C'4,2a enzyme; and they may eventually become of biological significance if applicable to enhancement of complement in vivo.

Materials and Methods

Preparations of the First Component of Human Complement.—C'1q, C'1r, and C'1s were prepared according to the method of Lepow (6). The esterase moiety of the first component (7) was isolated and kindly supplied by Dr. Irwin Lepow.

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* This is publication No. 228 from the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

‡ This work was supported by United States Public Health Service Grant AI-07007 and by American Heart Association Grant 65-G-166.

Preparation of the Second Component of Human Complement.—Approximately 800 ml of fresh serum was dialyzed for 48 hr against 3 × 10 liters of a 0.008 M solution of Na_3EDTA of pH 5.4 and a conductance of 1.25 millimhos per cm. The pseudoglobulin fraction thus obtained was separated from the euglobulin fraction by centrifugation and dialyzed overnight against phosphate buffer, pH 6.0, T/2 = 0.06, containing 0.002 M EDTA. This buffer, having a conductance of 4.0 millimhos per cm, will hereafter be referred to as "starting buffer." The dialyzed pseudoglobulin was then incubated at 37°C for 1 hr with diisopropyl fluorophosphate (DFP) at a final concentration of 10^{-3} M to inactivate an enzyme which, like C'1 esterase, has the ability to inactivate C'2 (and C'4) and which may, in fact, be identical with C'1 esterase. This enzyme was shown to be present in C'2-containing fractions obtained from carboxymethyl (CM) cellulose chromatography, and had previously been responsible for substantial inactivation of C'2 during early stages of its purification. The DFP-treated pseudoglobulin was then applied to a 7.5 × 60 cm column containing 2000 ml of packed CM cellulose, previously equilibrated with starting buffer. After the application of the sample, 10 liters of starting buffer was passed through the column; this process removed approximately 95% of the protein. C'2 was then eluted by the application of a gradient to 0.7 M NaCl in starting buffer. The fractions containing C'2 activity were pooled, dialyzed overnight against starting buffer, and resubjected to chromatography on CM cellulose under conditions similar to those used for the first chromatographic step, the only difference being that a 4.5 × 60 cm column was employed. This step resulted in a 4-fold purification and a 2-fold concentration of the material. The fractions containing C'2 activity were pooled and concentrated to approximately 8 ml. This sample, following further incubation with DFP, was subjected to preparative electrophoresis for 24 hr on a Pevikon block at 4°C in phosphate buffer, pH 6.0, T/2 = 0.05, containing 0.0025 M EDTA, and employing a potential gradient of 3.5 v/cm. The fractions containing C'2 activity were pooled and concentrated to approximately 5 ml. The C'2 at this stage in the isolation procedure had undergone an approximate 1800-fold purification in terms of protein, the recovery of C'2 hemolytic activity as determined by effective molecule titration (8) being approximately 30%. As a final step in the isolation procedure, this material was subjected to chromatography on OH-apatite, a step which will be described in detail elsewhere.[1] Throughout the present work, the preparation of C'2, concentrated following elution from the Pevikon block, was used as the source of C'2. The protein concentration was 1–1.5 mg/ml.

Preparation of the Third and Fourth Components of Human Complement.—C'3 and C'4 were isolated according to the method previously described (9, 10).

Preparation of EAC'1a, 4 Complex. 10 ml of a 2.5% suspension of sheep red cells sensitized with rabbit anti-sheep red cell antibody (EA) were incubated at 37°C for 15 min with 0.2 ml of each C'1q, C'1r, and C'1s, and 0.6 ml of saline containing 5.4 × 10^{-3} M Ca^{++} and 1.975 × 10^{-2} M Mg^{++}. 100 μg of purified C'4 was added and incubation continued for a further 15 min. The cells were washed twice with veronal buffered saline containing 1.5 × 10^{-4} M Ca^{++} and 4.8 × 10^{-4} M Mg^{++}, and then resuspended in the same buffer to their original volume, and in this state were referred to as EAC'1a,4.

Assays for C'2 Hemolytic Activity.—One procedure for the determination of C'2 activity was performed utilizing a serum reagent lacking C'2 (R_2) (11).

To determine C'2 activity more precisely, EAC'1a,4 cells were prepared as described above and the time of maximal formation of EAC'1a,4,2 a (t_{max}) at 32°C was determined both for untreated C'2 and iodine-treated C'2 (see below). 0.4 ml of EAC'1a,4 was incubated at 32°C with various amounts of C'2 mixed with an equal volume of the saline containing Ca^{++} and

[1] Polley, M. J., and H. J. Müller-Eberhard. 1967. Isolation of the second component of complement (C'2) from human serum. In preparation.

Mg^{++} ions. The total reaction volume was adjusted to 0.8 ml with veronal-NaCl buffer. At t_{max}, C'EDTA (0.1 ml fresh human serum +0.1 ml 0.2 M EDTA) was added to supply the six subsequent complement components and incubation was allowed to proceed for 30 min. The tubes were then transferred to an ice bath and 2 ml ice-cold normal saline was added to stop the reaction. The tubes were immediately centrifuged at 0°C and the supernatants were removed for spectrophotometric assay of the hemoglobin.

Kinetic Analysis of the Formation and Decay of EAC'1a,4,2a.—To 6 ml of a 2.5% suspension of EAC'1a,4, prewarmed to 32°C, was added 1 ml of a suitable amount of purified C'2, in which the chelating activity of the EDTA had been overcome by the addition of an appropriate amount of the solution containing free Ca^{++} and Mg^{++} ions. After thorough mixing, 0.6 ml aliquots were removed at intervals of time and transferred to a tube containing a mixture of 0.1 ml fresh human serum + 0.1 ml 0.2 M EDTA previously warmed to 32°C. After incubation for 30 min at 32°C, the tubes were transferred to an ice bath and processed as outlined above.

Preparation of the Fluid Phase Complex, (C'4i,2)a (C'3 Convertase).—This complex was prepared as described previously (1) utilizing for each preparation 200 μg of C'4 and amounts of C'2 varying between 1 and 100 μg. The reaction between C'1 esterase, C'4 and C'2 was allowed to proceed for 2 min at 32°C and then the material was stored at 0°C.

Treatment of (C'4i,2)a with Iodine.—Preparations of the complex were first treated with DFP at a final concentration of 5×10^{-3} M for 60 min at 0°C to inactivate C'1 esterase. This measure prevented formation of additional (C'4i,2)a from residual C'2 and C'4 subsequent to iodine treatment of the reaction mixture.

Each preparation of the complex was then divided into two equal parts, one of which was treated with an amount of iodine proportional to the quantity of C'2 used in its preparation; the complex prepared with 100 μg of C'2 was treated with iodine at a final concentration of 1.25×10^{-5} M in a 50-fold molar excess of KI. This reaction was allowed to proceed at 0°C for 15 min, after which time no free iodine was demonstrable by potassium iodide starch paper. The second part was not treated with iodine and served as the control.

C'3 Conversion, Its Detection and Quantitation.—100 μg of purified C'3 was added to each preparation of the complex. Following a 30 min period of incubation at 37°C, the conversion of C'3 to C'3i was stopped by cooling the reaction mixtures to 0°C. The degree of conversion was tested by immunoelectrophoresis and quantitated using radioactively labeled C'3 as described earlier (12).

Treatment of C'2 with Chemical Reagents.—One volume of the C'2 preparation was mixed with an equal volume of various concentrations of each reagent tested. The concentrations of the reagents ranged from 10- to 1,000-fold molar excess over the C'2 concentration. The reactions were allowed to proceed for 30 min at 4°C and were then terminated by dilution, dialysis, or passage through Sephadex.

RESULTS

Effect of Various Sulfhydryl Reagents on the Activity of C'2.—Since some degree of inactivation of C'2 encountered during its isolation procedure was prevented by the presence of EDTA, it was thought that the activity of C'2 might depend on a reactive sulfhydryl group within the molecule. This was particularly likely in view of Leon's observation that C'2 is inactivated by *p*-hydroxymercuribenzoate (13). In addition, it was found in this laboratory that treatment of C'2 with *p*-chloromercuribenzoate (*p*-CMB) and iodoacetic acid reduced the hemolytic activity to 10%, and with cyanogen bromide and *N*-ethylmaleimide to 60 and 80%, respectively. 2-Mercaptoethanol, dithiothreitol, cysteine, and glu-

tathione at concentrations greater than 10^{-3} M also caused substantial reduction of C'2 hemolytic activity. By contrast, treatment with iodoacetamide resulted in marked enhancement of C'2 activity. Further analysis of this phenomenon showed that iodoacetamide produced no such enhancement when used immediately following recrystallization. However, the ability to enhance the activity reappeared slowly on storage. It was, therefore, suspected that the enhancement was due to contaminating free iodine and not to iodoacetamide per se. This was, indeed, shown to be the case (Table I).

TABLE I

Effect of Various Sulfhydryl Reagents on the Hemolytic Activity of C'2

Reagent	Final concentration	Hemolytic activity (% of untreated C'2)
	M	
p-Chloromercuribenzoate	5×10^{-4}	10
Iodoacetic acid	5×10^{-2}	10
Cyanogen bromide	5×10^{-2}	60
N-Ethylmaleimide	5×10^{-2}	80
2-Mercaptoethanol	3×10^{-2}	48
Dithiothreitol	5×10^{-3}	38
Cysteine	3×10^{-2}	6
Glutathione	3×10^{-2}	14
Iodoacetamide (not recrystallized)	5×10^{-2}	800
Iodoacetamide (recrystallized)	5×10^{-2}	100
Iodine	2.5×10^{-5}*	1200

* Iodine diluted in 1.25×10^{-3} M potassium iodide.

Effect of Iodine Treatment of C'2 Hemolytic Activity.—Fig. 1 shows the effect of various concentrations of iodine at pH 6 on the hemolytic activity of C'2. As C'2 was found to be most stable at pH 6 and enhancement to be maximal at 2.5×10^{-5} M I_2 at pH 6, the following method was selected for iodine treatment of C'2. One volume of the C'2 preparation (approximately 1 mg/ml) was mixed with an equal volume of phosphate buffer, pH 6.0, T/2 = 0.1, containing 5×10^{-5} M I_2 and 2.5×10^{-3} M potassium iodide. The reaction mixture was kept for 5 min at room temperature before it was chilled to 0°C and suitably diluted for further use. The C'2 so treated will be referred to as ^{oxy}C'2.

It was possible under these conditions to achieve a marked enhancement of the hemolytic activity of C'2. Fig. 2 shows the results of a typical experiment to determine the dose response of unmodified C'2 and ^{oxy}C'2. y = per cent lysis and the negative logarithm of unlysed cells $[-\ln(1-y)]$ equals the average number of C'2a sites per cell (8). In the experiment depicted in Fig. 2, an average number of one site per cell was produced, when the relative concentration

of unmodified C'2 was 8.15 and that of ^{oxy}C'2, 0.5. The factor of enhancement was therefore 16.3. A similar degree of enhancement was obtained when the results were plotted according to the logarithmic transformation of the von Krogh equation (Fig. 3). The dose of untreated C'2 required for 50% hemolysis

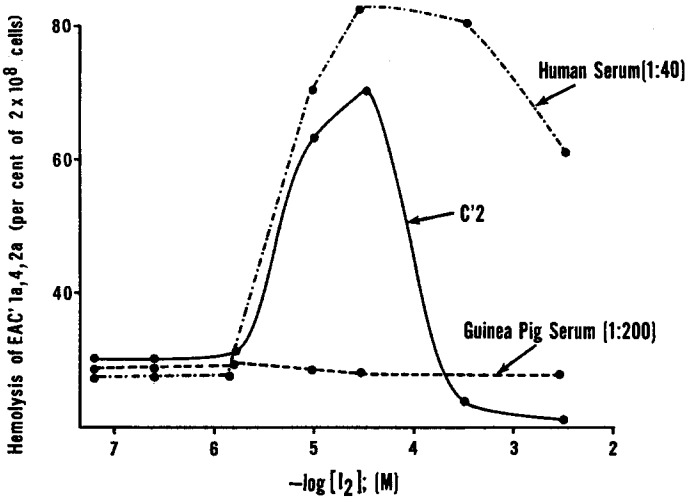

FIG. 1. The effect of increasing concentrations of iodine on the hemolytic activity of human C'2 contained in a partially purified preparation, and in whole human serum; and on guinea pig C'2 contained in whole guinea pig serum. Human serum was tested at a dilution of 1:40 and guinea pig serum at a dilution of 1:200.

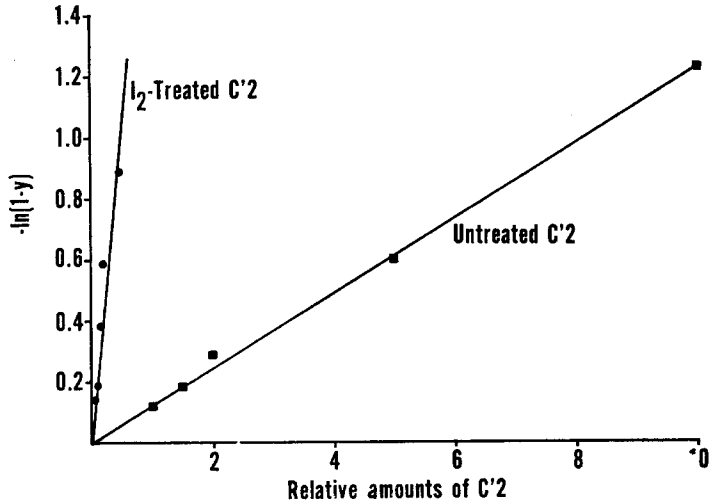

FIG. 2. Dose response for unmodified C'2, and iodine-treated C'2. Plotted as average number of C'2a sites per cell $[-\ln(1-y)]$.

($y/1\text{-}y = 1$) was 16.2 times that required of ^{oxy}C'2. Formation of EAC'1a,4,2a prepared with untreated C'2 is somewhat greater than that actually measured, the error being due to the lability of the complex. Considering the decay (8), the true extent of enhancement of C'2 activity by iodine is 13.6-fold.

The results of a typical experiment to determine the rate of formation and decay of the intermediate complex EAC'1a,4,2a prepared with untreated C'2 and chemically modified C'2 are shown in Fig. 4. In addition to the increase in apparent hemolytic activity of C'2 produced by iodine treatment, the use of

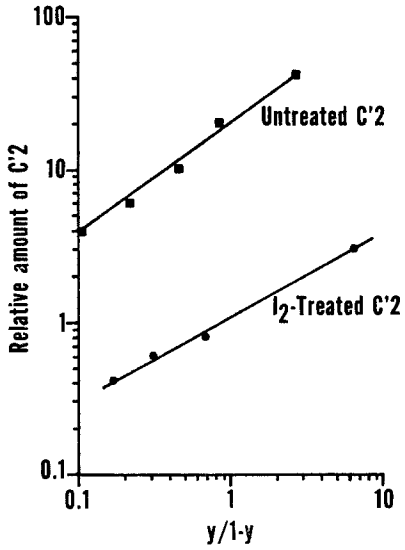

FIG. 3. Dose response for unmodified and iodine-treated C'2 plotted according to the logarithmic transformation of the von Krogh equation. y = per cent lysis; at 50% lysis $y = 0.5$ and $y/1-y = 1$ (reference 7).

^{oxy}C'2 had a marked effect on the half-life of EAC'1a,4,2a. Whereas the half-life of this complex at 32°C was 9 min when prepared with unmodified C'2, it increased to approximately 150 min when ^{oxy}C'2 was employed (Fig. 4). Further, EAC'1a,4,^{oxy}2a cells were found to be stable for several days when stored at 0°C. It was apparent from these results that the t_{max} was always increased to some extent when ^{oxy}C'2 was employed. Whereas the t_{max} utilizing unmodified C'2 varied only between 3 and 5 min, variations between 5 and 15 min were obtained with ^{oxy}C'2. Since t_{max} varies with the number of C'1a,4 sites per cell (8), a possible interpretation was that the iodine was, in fact, modifying the EAC'1a,4 cells. This was not the case, however, since iodine under the same conditions used for treating C'2 had no effect on EAC'1a,4, as measured by t_{max}. Furthermore, iodine treatment had no effect on either free solution or cell-bound C'4.

Another possible explanation for the observed enhancement was that iodine might reverse inactivation which had occurred during the process of isolation of C'2. That this was not the case is demonstrated in Fig. 1. Even fresh human serum exhibited a marked increase in C'2 hemolytic activity following treatment with iodine. Fresh guinea pig serum, however, exhibited no increase in C'2 hemolytic activity when tested under similar conditions. Similarly, no

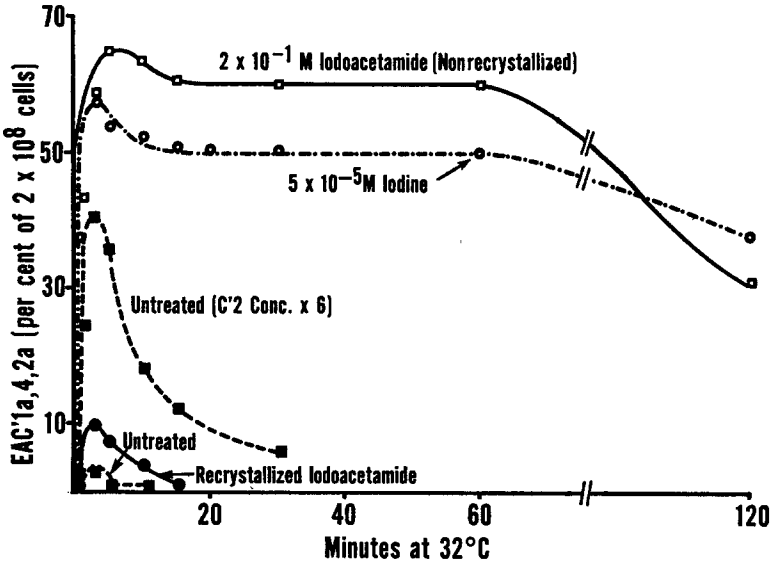

FIG. 4. Rate of formation and decay at 32°C of the intermediate complex EAC'1a,4,2a utilizing unmodified C'2 and C'2 following treatment with iodine, nonrecrystallized iodoacetamide, and iodoacetamide tested immediately following recrystallization. Untreated C'2 was tested at the same concentration as that used for the chemically modified C'2 and at six times this concentration. t_{max} for untreated C'2 was 3 min, 5 min for iodine-treated C'2, and 8 min for iodoacetamide-treated C'2.

increase in hemolytic activity was observed when whole sera from monkey, horse, and cow were tested following treatment with iodine.

Effect of Iodine Treatment on (C'4i,2)a.—An experiment to determine the effect of chemical modification of bound C'2 by treatment of EAC'1a,4,2a with iodine was not technically feasible, since iodine at the concentrations required produced lysis of the red cells. Therefore, the fluid phase (C'4i,2)a complex was utilized for this purpose. As shown in Table II, iodine treatment of preformed (C'4i,2)a caused an increase in the activity of this complex as measured by conversion of C'3. 90% conversion was achieved with untreated C'3 convertase prepared with 100 μg of C'2. A similar degree of conversion was observed with iodine-treated enzyme which was prepared with only 10 μg of C'2.

Effect of Sequential Treatment of C'2 with Sulfhydryl-Blocking Reagents and Iodine.—Table III shows the effects of treatment with iodine, *p*-CMB, and recrystallized iodoacetamide either singly or sequentially on the hemolytic activity of C'2. Recrystallized iodoacetamide has virtually no effect, nor does it prevent enhancement by iodine or inactivation by *p*-CMB. The finding

TABLE II

Effect of Iodine Treatment of (C'4i, 2)a on the C'3 Converting Activity of the Complex

Amount of C'2 used in preparation of (C'4i,2)a	% Conversion of C'3	
	(C'4i,2)a not treated with I_2	(C'4i,2)a treated with I_2
μg		
1	0	0
10	40	90
20	60	100
50	80	100
100	90	100

TABLE III

Effect of Treatment with Sulfhydryl Reagents, Either Singly or Sequentially, on the Hemolytic Activity of C'2

Sulfhydryl reagent		Hemolytic activity (% of untreated C'2)
First treament	Second treatment	
Iodine	—	810
Iodine	*p*-Chloromercuribenzoate	790
p-Chloromercuribenzoate	—	40
p-Chloromercuribenzoate	Iodine	780
Iodoacetamide*	—	110
Iodoacetamide*	Iodine	720
Iodoacetamide*	*p*-Chloromercuribenzoate	53

—, no further treatment with sulfhydryl reagent.

* Iodoacetamide was freshly recrystallized.

that treatment with iodine prevented subsequent inactivation by *p*-CMB suggested that both reagents affect and compete for the same critical group in C'2. However, treatment with *p*-CMB did not prevent subsequent enhancement by iodine. The latter observation appeared to contradict the above advanced hypothesis, unless under the conditions employed, the iodine was capable of removing the *p*-CMB. To find out whether it is principally possible to remove *p*-CMB from a sulfhydryl (-SH)- containing protein by treatment with iodine, experiments were performed using purified C'3, since this protein

is more readily available in larger quantities and has been shown to contain two free sulfhydryl groups.[2] Using identical conditions, C'3 was treated with ^{14}C-labeled *p*-CMB. After thorough dialysis, it was determined that 2 moles of *p*-CMB were bound per mole of protein. Subsequent treatment with iodine resulted in total release of the previously bound ^{14}C label (Table IV). Since in *p*-CMB the ^{14}C label is incorporated into the benzene ring, the most likely

TABLE IV

The Effect of Iodine Treatment on C'3 Previously Treated with ^{14}C-p-Chloromercuribenzoate

Treatment	Counts* per minute per 500 μg C'3
^{14}C-*p*-CMB	23,700
^{14}C-*p*-CMB; iodine	240

1 mg C'3 was treated with 10^{-3} M ^{14}C-*p*-CMB for 2 hr at 0°C. Following extensive dialysis, the material was divided into two equal portions, one of which was treated with 10^{-4} M I_2 in 5×10^{-3} M KI for 5 min at room temperature. Both aliquots were then subjected to further dialysis.

* Counts were corrected for quenching and efficiency of counting by the liquid scintillation counter.

TABLE V

Iodine-Induced Enhancement of C'2 Hemolytic Activity Compared with Uptake of Iodine

pH	C'2 Hemolytic activity (% of untreated C'2)	Uptake of ^{125}I
		*cpm/100 μg**
5	254	142,570
6	440	118,296
8	110	112,914

* Preparation of partially purified C'2.

interpretation of the total removal of counts by the iodine treatment is that under the conditions used the *p*-CMB was removed from the C'3 molecules.

The Nature of the Effect of Iodine Causing Enhancement of C'2 Activity.—The results presented above suggested that the iodine effect on C'2 activity was due to the reaction of iodine with a sulfhydryl group in the C'2 molecule. Further evidence in support of this view was obtained through ^{125}I-uptake studies. C'2 was treated with ^{125}I at three different pH's and uptake of iodine was compared with enhancement of activity. Table V shows that whereas enhancement of activity was pH dependent, being maximal at pH 6, uptake of ^{125}I was no greater at pH 6 than at the other two pH values. Conditions for the uptake experiment were such that for every 2 moles of radioactive iodine, 10^4 moles

[2] Polley, M. J., and H. J. Müller-Eberhard. Unpublished observations.

unlabeled iodide and 10^3 moles unlabeled iodine were present. Provided that no protein in the preparation was labeled preferentially under these conditions and assuming a molecular weight of 115,000 for C'2 (1), it was calculated that at maximal enhancement of C'2 hemolytic activity, one mole of C'2 had bound less than 0.05 mole of iodine. It was, therefore, concluded that the iodine effect was not due to uptake of iodine, but to oxidation of some group in the C'2 molecule. Further proof for oxidation as opposed to iodination was obtained through the observation that the reducing agent, sodium dithionite, reversed the iodine effect and lowered the activity of treated C'2 to the original level or even below that of untreated C'2. Moreover, subsequent treatment with iodine resulted in renewed enhancement of activity (Table VI).

TABLE VI

Effect of Sequential Treatment with Iodine and the Reducing Agent Sodium Dithionite on the Hemolytic Activity of C'2

Chemical reagent			Hemolytic activity (% of untreated C'2)
Treatment 1	Treatment 2	Treatment 3	
Iodine	—	—	225
Iodine	Iodine	—	6
Iodine	Sodium dithionite	—	20
Iodine	Sodium dithionite	Iodine	230

—, no further treatment.

DISCUSSION

Treatment of C'2 with a critical concentration of iodine has been shown to result in a more than 10-fold enhancement of its hemolytic activity and in the stabilization of the intermediate complex EAC'1a,4,2a prepared utilizing the chemically modified C'2. The precise nature of the induced chemical change and of the groups affected has not been defined. However, it was possible to eliminate certain explanations of the effect in preference to others. Thus, the principal reaction involved in iodine treatment of proteins is substitution into the tyrosyl residues resulting in the production of the monoiodo- or diiodotyrosyl derivative of the protein (14). In the present study, under conditions favoring maximal enhancement of the hemolytic activity of C'2, 0.05 mole of iodine was bound per mole of protein. Substitution into the tyrosyl residues as the cause for enhancement of C'2 activity therefore seemed very unlikely. For the same reason, substitution into the imidazole ring of histidine appeared an equally unlikely explanation. Further evidence against histidine substitution being responsible for the iodine effect is the speed with which the enhancement became apparent. Whereas iodine substitution into histidine is a time dependent mechanism (14), the effect of iodine on C'2 is apparently instan-

taneous. By a process of elimination, therefore, oxidation of C'2 was considered to be the most probable interpretation of the mechanism of enhancement. Since sulfhydryls are the most susceptible groups in proteins to oxidation by iodine, it is postulated that it is this type of group that is primarily involved in the observed activity change, although oxidation of a functionally relevant carbohydrate group has not been ruled out.

The oxidation of a sulfhydryl group takes place by two main routes. Mild oxidation of simple thiols results in the formation of disulfides. When strong oxidizing agents are employed, the reaction can proceed via sulfenic (-SOH) through sulfinic ($-SO_2H$) to the sulfonic acid stage ($-SO_3H$). The actual mechanism of the reaction and the intermediates involved are dependent on the conditions employed (13). Two sulfhydryls can be oxidized to form a disulfide provided they are not sterically hindered in doing so (16). In a protein molecule with relatively few -SH groups present, it may be impossible for any two of them to form a disulfide bond. Such an example is provided by β-lactoglobulin. Treatment with iodine results in conversion of its two -SH groups to sulfenyl iodide and even in the presence of strong oxidizing agents these groups fail to form a disulfide bond. The behavior of the sulfhydryl groups in ovalbumin is even more complex. Of the four -SH groups present in the native protein, only three are reactive towards iodine. These are oxidized to sulfenyl iodides possessing varying degrees of stability, one being almost instantaneously decomposed to sulfenic acid, while the other two remain stable for several days (17).

Since the poor uptake of iodine by C'2 indicates lack of substitution, formation of a sulfenyl iodide can also be excluded. Disulfide bond formation appears to be precluded by the finding that the iodine effect is reversible by treatment with $Na_2S_2O_4$, a reducing agent which is not known to split disulfide bonds. However, oxidation of a disulfide bond by iodine with the resulting formation of two sulfonic acid derivatives (15) cannot be excluded at present as a possible mechanism of enhancement. Nevertheless, the most likely interpretation is that the reaction of C'2 with iodine results in oxidation of one or more sulfhydryl groups to one of the sulfonic acid derivatives. Whether it be the sulfenic, sulfinic, or sulfonic form remains to be determined. Reversal of the iodine effect by the reducing agent sodium dithionite lends further support to this view.

Sequential treatment of C'2 with iodine and sulfhydryl reagents led to the apparently contradictory observations that whereas prior treatment with iodine prevented subsequent inactivation by *p*-CMB, prior treatment with *p*-CMB did not prevent subsequent enhancement by iodine. These findings were compatible only if iodine under the conditions employed could remove *p*-CMB from the critical sulfhydryl group. That this interpretation was indeed likely was shown by the finding that treatment with iodine under identical conditions to those used with C'2 totally removed the ^{14}C label from C'3 previously substituted with ^{14}C-*p*-CMB.

Enhancement of the activity of an enzyme by treatment with iodine is not unique. A 5-fold increase in the esterase activity of carboxypeptidase A following treatment with iodine has been reported (18). However, in this case the iodine-induced enhancement was shown to be the result of iodine substitution into two reactive tyrosyl residues closely associated with the active center of the enzyme (19).

In the present communication, data are presented describing the iodine-induced enhancement of another enzyme: C'3 convertase. This enzyme, which occurs in free solution and in cell-bound form, has recently been shown to be composed of two subunits, one derived from C'2 and the other from C'4 (1). In contrast to carboxypeptidase A, the effect of iodine on C'3 convertase appears to be due to oxidation of one or more critical sulfhydryl groups. The accumulated evidence indicates that the increase in activity and stability of the enzyme results solely from the modification of its C'2 subunit. The critical modification can be affected by treating native C'2 before it enters into the complex with C'4 or by treating the C'4,2a complex. Since both activity and stability of the enzyme are affected, one might postulate that two distinct groups in the C'2 portion are involved, one located in the active site, the other in the combining region. Alternatively, one could visualize the iodine effect to be caused by the modification of only one critical group which allosterically controls the active as well as the combining site. Further elucidation of the iodine effect will have to await the preparation of larger amounts of highly purified C'2 for chemical analyses, a task which is rendered difficult by the exceedingly low concentration of C'2 in serum.

SUMMARY

A method has been described for the chemical modification of human C'2 which results in a pronounced enhancement of its hemolytic activity and a marked increase in the stability of the intermediate complex EAC'1a,4,2a prepared with the modified C'2. Both effects are fully explained by the observed increase in activity and stability of the C'3 converting enzyme, C'4,2a, following its generation with modified C'2. Evidence has been presented in support of the hypothesis that the modification resulting from treatment of C'2 with a critical concentration of iodine consists of oxidation of one or more sulfhydryl group within the molecule.

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