Vol. 107, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1982 Pages 618-623

THE DISSOCIATION PROPERTIES OF NATIVE C1

Robert J. Ziccardi[a] and Jurg Tschopp

Department of Molecular Immunology

Research Institute of Scripps Clinic

La Jolla, California 92037

Received June 17, 1982

The first component of human complement (C1) readily dissociates under physiologic conditions into two subunits - C1q and $C1r_2C1s_2$. The equilibrium constant for this reaction has been determined for native C1 in fresh normal human serum by hemolytic titration. Standard technology was modified to simulate physiologic conditions. Furthermore, assays were carried out at numerous concentrations of sensitized erythrocytes, thereby allowing the calculation of the percent of associated C1 at different total C1 concentrations. Increased C1 dissociation was observed with dilution. From these data, an association constant of 4.5×10^8 M^{-1} was calculated for native C1. Thus in normal human serum approximately ten percent of the C1 is present as free C1q and $C1r_2C1s_2$.

INTRODUCTION

The first component of human complement (C1)[b] dissociates in the presence of EDTA into three distinct subunits - C1q, C1r and C1s (1,2). However under physiologic conditions, the dissociation products are primarily C1q and $C1r_2C1s_2$. The equilibrium constant for the latter reaction has been calculated for C1 reconstituted from purified proteins (3,4) and for C1 having had one subunit (C1s) reincorporated after having been purified and radiolabeled (4).

An understanding of the dissociation properties of C1 is important for the proper analysis of C1 activation kinetics (4,5). Furthermore, increased concentrations of

[a] This is publication number 2746 from the Research Institute of Scripps Clinic. This work was supported by USPHS grants AI 14502 and AI 17354. Dr. Ziccardi is the recipient of Established Investigatorship 79-139 from the American Heart Association. Dr. Tschopp is supported by the Swiss National Science Foundation.

[b] Abbreviations: AVB, veronal-buffered saline at physiologic ionic strength (0.15 M); C1, first component of human complement; EAC4, antibody sensitized sheep erythrocytes bearing functionally active human C4; EAC14, antibody sensitized sheep erythrocytes bearing functionally active human C1 and C4; EDTA, ethylenediaminetetraacetic acid; K_{assoc}, association constant; NHS, normal human serum; SAVB, veronal-buffered saline at reduced ionic strength (0.065 M).

0006-291X/82/140618-06$01.00/0

free $C1r_2C1s_2$ and C1q have been detected in certain pathological sera (6). Therefore we have investigated the dissociation properties of native C1 in normal human serum. An association constantof 4.5 x 10^8 M^{-1} has been calculated for native C1 indicating that approximately 10 percent of the C1 in normal human serum is dissociated into C1q and $C1r_2C1s_2$. Furthermore, we can conclude that C1 reassembled from purified components is almost as stable as native C1 and thus can be used as a model for the study of the biochemical and biophysical characterization of the activation of C1.

MATERIALS AND METHODS

Normal Human Serum. Blood was drawn from healthy human donors and clotted 1 hour at room temperature. The clot was allowed to retract in ice for 30 minutes after which serum was obtained by centrifugation and kept on ice until used on the same day.

C1 Hemolytic Titration. The method of Borsos and Rapp (7) for the hemolytic titration of C1 was used with the following changes: 1. In order to determine the association constant of C1 under physiologic conditions, C1 was diluted and incubated with indicator cells in a buffer of physiologic ionic strength (0.15 M) instead of reduced ionic strength (0.065 M). 2. Buffers contained 0.1 percent bovine serum albumin to prevent nonspecific protein loss at the high dilutions utilized (gelatin was avoided due to its potential to interact with the structurally homologous C1q).

In brief, hemolytic titration of C1 was carried out as follows: 1. Normal human serum, NHS, was diluted in AVB (i.e. veronal-buffered saline containing 0.1 percent albumin, 0.00015 M calcium, 0.001 M magnesium, pH 7.5, μ = 0.15M) and incubated at 30°C for 5 min. 2. 0.2 ml of diluted NHS was mixed with 0.1 ml EAC4 (i.e. sensitized erythrocytes bearing active C4) in AVB and incubated at 30°C for 30 min. 3. These EAC14 were washed once with the cells taken up in 0.3 ml of a low ionic strength buffer - SAVB (i.e., isotonic veronal-buffered saline containing 4 percent sucrose, 0.1 percent albumin, 0.00015 M calcium, 0.001 M magnesium, pH 7.5, μ = 0.065M). The low ionic strength prevents "C1 transfer" in the next reaction step (7). 4. An excess of oxidized C2 in 0.1 ml SAVB was next added and the mixture incubated at 30°C for 10 minutes. 5. Finally 1.1 ml of guinea pig serum (diluted 1:50 in veronal buffer containing 0.04 M EDTA) was added and the mixture was incubated at 37°C for 60 minutes (note that for the highest cell concentration utilized (Table I), guinea pig serum was diluted 1:20 in order to be in excess). Hemoglobin released from lysed cells was measured by absorbance at 412 mμ after spinning out unlysed cells. The reciprocal of serum dilution was plotted against - ln (1 - percent lysis). The dilution at which - ln (1 - percent lysis) equalled unity corresponded to that quantity of C1 required to initiate the lysis of 63 percent of the offered cells.

RESULTS

The concentration of functionally active C1 in fresh normal human serum was determined by hemolytic titration as described in the Materials and Methods section. In five separate experiments, the number of added indicator cells (i.e., EAC4) was varied from 1.3 x 10^7 to 24 x 10^7. For each cell dose, the serum dilution yielding enough C1 to initiate the lysis of 63 percent of the offered cells

TABLE I

HEMOLYTIC TITRATION OF C1 IN NHS

Number of EAC4	Serum dilution for 63% lysis[a]	Associated C1 Molecules[b]	Total C1 Molecules[c]	Percent Associated C1[d]
1.27×10^7	6,900	1.27×10^7	3.1×10^9	0.41%
2.80×10^7	5,100	2.80×10^7	4.2×10^9	0.67
6.50×10^7	4,000	6.05×10^7	5.4×10^9	1.1
12.1×10^7	3,550	12.1×10^7	6.1×10^9	2.0
23.9×10^7	2,400	23.9×10^7	9.0×10^9	2.7

[a]Serum dilution such that there is enough C1 in 0.2 ml to initiate the lysis of 63% of the offered cells (column 1).

[b]Equals the number of EAC4 (column 1) and represents the hemolytically active C1 molecules in 0.2 ml of serum diluted as shown in column 2.

[c]Total number of C1 molecules in 0.2 ml of serum diluted as shown in column 2. Total C1 concentration (immunochemically determined) in undiluted NHS was taken as 1.8×10^{-7} M (10,11).

[d]Equals column (3) ÷ column (4) x 100.

was determined (Table I, column 2). According to the Poisson distribution function, there is one active C1 per cell at this dilution since C1 initiated hemolysis follows the one-hit theory of lysis (7-9). Thus the number of active C1 molecules (i.e., associated C1) (Table I, column 3) is simply equal to the number of EAC4. The total number of C1 molecules present at this dilution (Table I, column 4) was calculated from the immunochemically determined serum C1 concentration of 1.8×10^{-7} M (10,11). Finally, the percent of associated C1 was readily calculated from these figures for each experiment (Table I, column 5). As expected, the percent of associated C1 decreased with greater dilution.

In order to determine the association constant, the experimental data from Table I were plotted as percent associated C1 versus total C1 concentration and then compared to theoretical curves calculated assuming various association constants (Fig. 1). The following equation was used to generate the theoretical curves:

$$X = 100 \times \left[\frac{2 \times K_{assoc} \times [C1]_t + 1 - \sqrt{4 \times K_{assoc} \times [C1]_t + 1}}{2 \times K_{assoc} \times [C1]_t} \right] \quad (1)$$

where X = percent associated C1 and $[C1]_t$ = total C1 concentration.

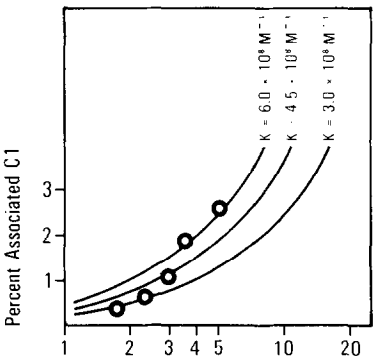

Figure 1. Determination of the association constant of C1 in NHS. The percent of associated C1 (from Table I, column 5) is plotted versus total C1 concentration (Table I, column 4 ÷ 0.3 ml ÷ 6×10^{23}). Open circles represent experimental points. Solid lines represent theoretical curves generated from equation (1) assuming numerous association constants.

The experimental data are consistent with a K_{assoc} of 3×10^8 M^{-1} to 6×10^8 M^{-1}. Using a mean value of 4.5×10^8 M^{-1} for K_{assoc} and 1.8×10^{-7} M for $[C1]_t$ and equation (1), we can conclude that approximately ten percent of the C1 in NHS is present as free C1q and $C1r_2C1s_2$.

DISCUSSION

An association constant of 4.5×10^8 M^{-1} has been calculated for native C1 under physiologic conditions. Thus in normal human serum, approximtely 10 percent of the C1 is present as free C1q and $C1r_2C1s_2$. Standard hemolytic technology (7) was utilized for these studies with the few modifications described in the Materials and Methods section. For calculations it was assumed that all macromolecular or associated C1 was hemolytically active, while free C1q and $C1r_2C1s_2$ were hemolytically inactive. Evidence in favor of this assumption includes the finding that all associated C1 was activateable while dissociated C1 was not when assayed by gel analysis (5).

It is readily apparent from the data in Table I (column 5) that only a small percent of the serum C1 is detected by hemolytic titration. This is due to dissociation at the high dilutions required for this assay. While the standard hemolytic titration is carried out at reduced ionic strength (7), thereby increasing associated C1, still less than six percent of the serum C1 is associated and therefore titrateable. This percent was calculated from the total serum C1

TABLE II
C1 ASSOCIATION CONSTANTS

C1 source	K_{assoc}	Methodology	Percent of C1 Associated in NHS[b]	Reference
Native C1 in NHS	4.5×10^8 M^{-1}	hemolytic titration	89%	present study
Reconstituted C1	6.7×10^7	ultracentrifugation	75%	Siegel et al. (3)
Exchanged C1	2.0×10^7[a]	ultracentrifugation	60%	Kilchherr et al. (4)

[a]Reported as a lower limit.

[b]Calculated from K_{assoc} and equation (1) with $[C1]_t = 1.8 \times 10^{-7}$ M.

concentration (immunochemically determined to be 1.8×10^{-7} M (10,11) and the titrateable C1 concentration (6×10^{12} C1/ml or 1.0×10^{-8} M (10)).

Association constants have been previously calculated for C1 reconstituted from purified proteins (3,4), and for C1 having had one subunit (C1s) reincorporated after having been purified and radiolabeled (i.e., exchanged C1) (4). These values as well as that calculated in this study for native C1, are listed in Table II. There is reasonable agreement in K_{assoc} although native C1 is somewhat more stable than reconstituted or exchanged C1 (it should be noted that the K_{assoc} for exchanged C1 was reported as a lower limit). From these association constants, we calculate that at physiologic concentrations, 89 percent of native C1 and 75 percent of reconstituted C1 is associated (Table II) . Thus reconstituted C1 can be used as a model for the study of the biochemical and biophysical characterization of the activation of C1.

ACKNOWLEDGEMENTS

We wish to thank Deborah Casbero and Julie Harper Ziccardi for expert technical assistance and Ellye Lukaschewsky for secretarial services.

REFERENCES

1. Lepow, I.H., Naff, G.B., Todd, E.W., Pensky, J. amd Hinz, C.F. (1963) J. Exp. Med. 117, 983-1007.
2. Ziccardi, R.J. and Cooper, N.R. (1978) Science 199, 1080-1082.
3. Siegel, R.C., Strang, C.J., Phillips, M.L., Poon, P.H. and Schumaker, V.N. (1981) Fed. Proc. 40, 963 (Abstract).
4. Kilchherr, E., Fuchs, H., Tschopp, J. and Engel, J. (1982) Mol. Immunol. 19, 683-691.
5. Ziccardi, R.J. (1982) J. Immunol. 128, 2500-2504.

6. Laurell, A.B., Martensson, U., and Sjoholm, A.G. (1976) Acta Path. Microbol. Scand. Scet. C. 84, 455-464.
7. Borsos, T. and Rapp, H.J. (1963) J. Immunol. 91, 851-858.
8. Rapp, H.J. (1955) Mathematical Theory and Analysis of Immune Hemolysis. Dissertation. The Johns Hopkins University School of Hygiene and Public Health.
9. Colten, H.R., Borsos, T. and Rapp, H.J. (1967) Science 158, 1590-1592.
10. Ziccardi, R.J. and Cooper, N.R. (1977) J. Immunol. 118, 2047-2052.
11. Ziccardi, R. J. (1981) J. Immunol. 126, 1769-1773.