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FLUOROMETRIC DETECTION OF LOW TEMPERATURE THERMAL TRANSITIONS IN THE C1Q COMPONENT OF HUMAN COMPLEMENT

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SUMMARY: Fluorescence studies with the human complement component C1q were performed as a function of temperature and demonstrated the existence of low temperature, thermally induced structural transitions in the C1q molecule. Both intrinsic protein fluorescence and the fluorescence of the apolar probe 2-p-toluidinylnaphthalene-6-sulfonate independently showed thermal transitions at 15°C, 35°C and 48°C. C1q activity measurements indicated no loss of hemolytic activity at temperatures below 46°C. It is proposed that these structural transitions are a consequence of the internal flexibility of the native C1q molecule.

INTRODUCTION

The interaction of the blood protein C1q with immune complexes activates a highly specific cascade of eleven plasma proteins, which together with C1q represent the components of the classical complement pathway (1). A wide variety of biological activities are expressed upon complement activation including the lysis of cells to which immunoglobulins are specifically bound. Since C1q binding to immune complexes can trigger the complement cascade in the absence of detectible covalent modifications in the C1q molecule, it has been proposed that classical pathway activation is dependent upon conformational changes in the C1q structure (1,2,3). However, previous studies (4,5) have failed to demonstrate C1q conformational changes that could be considered directly relevant to the complement activation process. We report here the existence of low temperature, thermally induced conformational changes in the C1q molecule which are demonstrable by fluorescence spectroscopic procedures. We propose that rearrangements of the internal structure of the C1q molecule are an essential requirement for the expression of its role as the bridge

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between immunoglobulins and one of their most important effector systems, the complement sequence.

METHODS

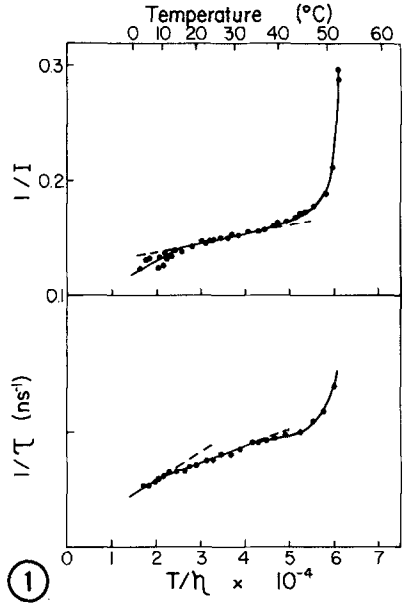
Clq was isolated from human serum by a procedure involving affinity chromatography as previously described (6). Clq activity was measured at 37°C in a hemolytic assay using a Clq-depleted reagent and sensitized sheep erythrocytes (6).

Fluorescence measurements were made on an SLM Model 4800 spectrofluorometer (Urbana, Illinois). For intrinsic protein fluorescence, excitation was 295 nm and emission was monitored at 344 nm. The fluorescence of 2-p-toluidinylnaphthalene-6-sulfonate (TNS) (Molecular Probes; Plano, Texas) was monitored at the emission maximum when excited at 366 nm. Fluorescence lifetimes were measured on a phase-modulation cross-correlation SLM fluorometer and all values are from phase lifetime measurements with the exciting light modulated at 30 MHz.

The cuvette temperature in the fluorescence measurements was controlled by circulating water and after each temperature change 10 minutes was allowed for thermal equilibrium to be obtained.

RESULTS AND DISCUSSION

Bushueva et al. (7) have shown that the fluorescence intensity (I) and intrinsic fluorescence lifetime (τ) of the tryptophans in proteins depend on the solution viscosity (η), and the absolute temperature (T). A smooth function with no breaks is predicted when either $1/\tau$ or $1/I$ is plotted as a function of T/η. This behavior occurs because of the temperature dependence of fluctuations in the protein structure surrounding the fluorescent residues. Each distinct conformational state is expected to be characterized by a distinct $1/\tau$ or $1/I$ versus T/η plot. These expectations, that different smooth plots will be displayed by different native conformations, have been verified for more than twenty native globular proteins (7). These considerations also lead to the expectation that thermally-induced transitions which interconvert protein conformers that differ in their flexibility near tryptophan residues, e.g., changes in intramolecular domain-domain interations or contacts, will be characterized by plots of $1/I$ or $1/\tau$ vs T/η that have breaks separating smooth segments (8).



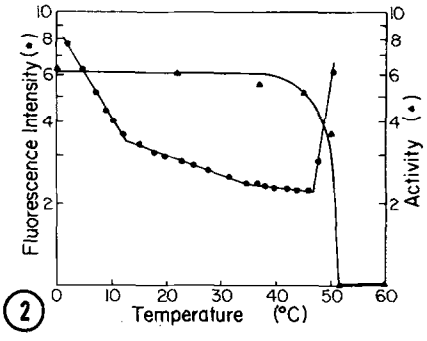


Figure 1. Reciprocal of the intrinsic fluorescence intensity of Clq ($1/I$, upper panel) and the reciprocal of the fluorescence lifetime of Clq ($1/\tau$, lower panel) as a function of the temperature over viscosity ratio (T/η) obtained by varying solution temperature. The Clq concentration was 0.2 mg/ml for the intensity measurements and 0.4 mg/ml for the lifetime measurements. All measurements were performed in 0.01 M Tris HCl buffer. pH 7.4 containing 50 mM NaCl.

Figure 2. Temperature dependence of Clq activity (▲) and the fluorescence of 2-P-toluidinylnaphthalene-6-sulfonate (TNS) (●). For the activity measurements, samples were incubated at the indicated temperature for times comparable to the fluorescence measurements and assayed at the common temperature of 37°. TNS measurements were performed in 0.01 M Tris-HCl buffer, pH 7.9 containing 50 mM NaCl. Clq concentration was 0.24 mg/ml and the TNS concentration was 7.0×10^{-5} M.

Figure 1 shows these plots for highly purified human Clq (6) when T/η is varied by changing the temperature. Three thermal transitions are observed which occur at approximately 15°C, 35°C and 48°C. Figure 2 shows the activity of Clq in a hemolytic assay (6) conducted at 37°C after preincubation of the protein over the same temperature range as in Figure 1 and indicates that no irreversible conformational changes occur in Clq below a temperature of approximately 46°C.

Toluidinylnapthalene-6-sulfate (TNS) is one of a class of compounds which are weakly fluorescent in water but become highly fluorescent when bound to apolar sites on proteins. Figure 2 shows that TNS binding reveals the same three thermal transitions as reported by the intrinsic

fluorescence studies. Since these two methods are independent indicators of protein conformational characteristics, the results clearly indicate that structural changes in C1q are responsible for the observed thermal transistions. The transition observed at temperatures higher than 45°C correspond to the irreversible loss of functional activity as a result of denaturation and has been reported previously in other studies (9).

Electron microscopy in conjunction with analysis of the fragments produced upon limited proteolytic digestion of C1q have led to the "bouquet of tulips" model for C1q in which 6 collagen-like stem regions are joined at one end in a central core and terminated at the other end by a globular portion or head region (10,11). This model of the C1q structure requires a 6-fold symmetry of the head groups and implies the head regions independently interact with immunoglobulins. Another implicit feature of this model is that the C1q molecule can be represented by a static structure which remains essentially rigid during the complement activation reaction. This model of an inflexible C1q molecule has been supported by circular dichroism studies which reported no changes in C1q average secondary structure as a function of temperature (4). In contrast, the results presented here indicate the C1q molecule is flexible, exhibiting low temperature, thermally induced structural transitions. Since the tryptophan residues in C1q are located in the globular head regions (1,12), these thermal transitions must represent conformational alterations which affect the head regions. The data presented would be compatible with the conclusion that C1q is capable of adopting a conformation(s) in solution in which internal interactions are possible involving both head-head and stem-stem contacts and the observed transitions would relate to the alteration of these contacts. The low energy of these conformational changes would allow them to be modulated by the known interactions with immunoglobulins in which C1q participates. Such low energy thermal transitions ($\sim$22°C) have recently been observed with photon correlation spectroscopic studies

of hemoglobin (13) and have been ascribed to quaternary rearrangements in the hemoglobin molecule. Consistent with this conclusion, C1q initiation of the classical complement pathway would involve changes in the mutual orientation among head groups and stems. Since these changes would result in only small perturbations in the average secondary structure of the molecule, they would be undetectable by methods previously employed by other investigators (4).

Examination of the primary amino acid sequences of the three C1q subunit chains (A,B, and C) indicates the Gly-X-Y repeating triplet of the collagen-like sequence is disrupted at position 36 in the C chain where alanine is substituted for glycine (14). This substitution results in a bend approximately half-way through the C1q collagen-like regions at the point where the central core joins the outstretched and extended connecting strands. This break in the helical stem structure might allow this region to exhibit hinge-like properties and would be a likely site to account for the observed internal flexibility of the C1q molecule.

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REFERENCES

1. Porter, R.R. and Reid, K.B.M. Nature 275, 699-704 (1978).
2. Müller-Eberhard, H.J. Ann. Rev. Biochem. 38, 389-414 (1969).
3. Reid, K.M.B. and Porter, R.R. in Contemporary Topics in Molecular Immunology (Inman, F.P. and Mandy, W.J., ed.) Vol. 4, pp. 1-20, Plenum Press, New York.
4. Brodsky-Doyle, B., Leonard, K.R., and Reid, K.B.M. Biochem. J. 159, 279-286 (1976).
5. Paul, S.M., Baillie, R.D. and Libertic, P.A. J. Biol. Chem. 253, 5658-5664 (1978).
6. Kolb, W.P., Kolb, L.M. and Podack, E.R. J. Immunol. 122, 2103-2111 (1979).
7. Bushueva, T.L., Busel, E.P. and Burstein, E.A. Biochim. Biophys. Acta 534, 141-152 (1978).

8. Waslewski, Z. and Horowitz, P. Fed. Proc. 39, 791 (1980).
9. Lowe, D.M. and Reid, K.B.M. Biochem. J. 143, 265-272 (1974).
10. Shelton, E., Yonemasu, K. and Stroud, R.M. Proc. Nat. Acad. Sci. USA 69, 65-68 (1972).
11. Reid, K.B.M. and Porter, R.R. Biochem. J. 155, 19-23 (1976).
12. Reid, K.B.M. and Thompson, E.O.P. Biochem. J. 173, 863-868 (1978).
13. Beldkamp, W.B. and Votano, J.R. Biopolymers 19, 111-124 (1980).
14. Reid, K.B.M. Biochem. J. 161, 247-251 (1977).