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SEGMENTAL FLEXIBILITY OF IMMUNOGLOBULINS

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One of the major functions of antibody is to initiate biological effector functions, such as complement activation, upon binding to antigen. To fulfill these functions, antibody molecules have evolved into a set of related structures with unusual conformations and appropriate dynamic properties. Perhaps the most intriguing feature is the folding of the light and heavy chains into separate and compact domains with homologous conformations (1).

We have used nanosecond fluorescence depolarization measurements of fluorescent hapten-antibody complexes and proteolytic fragments to explore the nature of subunit motions of IgM class antibodies. These molecules are pentamers of IgG-like subunits which, however, contain an additional domain, called Cμ2, between Fab and $(Fc)_5$ in each heavy chain (2).

In native, intact IgM, the Fab moieties were found to display restricted segmental flexibility (3). Heating the IgM to 60° for 30 min significantly reduced the ability to fix or activate complement in the presence of antigen, although binding sites for C1q were still present and the antibodies still bound antigen. Depolarization measurements of the heated IgM and of $(Fab')_2\mu$ fragments showed that segmental flexibility had increased (Table I).

At the same time, a comparison of the circular dichroism of native and heated IgM, Fab, and $(Fab')_2\mu$ fragments showed that a localized alteration of the Cμ2 domains had occurred. However, specific rabbit antibodies elicited against the Cμ2 region of native $(Fab')2\mu$ fragments could still bind to the heated IgM or $(Fab')_2\mu$. Taken together, these results suggest that the interaction between paired Cμ2 domains in each IgM_5 subunit had been altered by exposure to heat.

Similar, although more extensive, changes occurred on exposure of the IgM to 1 M acetic

TABLE I

NANOSECOND FLUORESENCE ANISOTROPY OF IgM AND $(Fab')_2\mu$. SUMMARY OF THE PARAMETERS OBTAINED BY FITTING THE ANISOTROPY TO A SUM OF TWO EXPONENTIALS $[(A(t) = A_0(f_s e^{-t/\phi_s} + f_L e^{-t/\phi_L})]$ BY A WEIGHTED NONLINEAR LEAST-SQUARES ANALYSIS

Dansyl-lysine-anti-dansyl complex	A_0	t^*_{max}	f_s	ϕ_s	f_L	ϕ_L
		(*ns*)		(*ns*)		(*ns*)
Native IgM	0.248 ± 0.002	150	0.49 ± 0.01	34 ± 2	0.51 ± 0.01	241 ± 7
Heat-treated IgM	0.246 ± 0.002	150	0.62 ± 0.08	39 ± 7	0.38 ± 0.08	243 ± 76
Native $(Fab')_2\mu$	0.254 ± 0.005	115	0.30 ± 0.07	22 ± 4	0.70 ± 0.07	85 ± 9
Heat-treated $(Fab')_2\mu$	0.250 ± 0.005	115	0.60 ± 0.07	30 ± 4	0.40 ± 0.07	93 ± 11

*Longest time from which *A(t)* data points were fit.

acid followed by neutralization. Electron micrographs of our preparations confirmed these results.[1]

Because we have found that activation of complement by native IgM appears to require binding to multivalent antigens in which the spacing of determinants is critical, and is not due simply to aggregation of IgM molecules, our results can be interpreted to mean that binding of several Fab moieties within a given IgM to antigen either causes a conformational change within $(Fc)_5$, or freezes a particular conformation, a process which must be mediated by the intervening $C\mu 2$ domains.

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FLUORESCENCE ENERGY TRANSFER BETWEEN PORCINE PEPSIN AND DANSYL-PEPTIDE INHIBITOR

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Activation of porcine pepsinogen by exposure to low pH leads to the release of the 44 amino terminal residues in the form of several peptide fragments (1). Peptide (1–16) has been shown to be a strong inhibitor of the proteolytic activity of pepsin at pH 5.5, with K_I of 0.2 μM. We have prepared several analogs of this sequence by solid phase peptide synthesis to examine the critical functional residues for this inhibition.

METHODS

Peptide synthesis was accomplished by the solid phase method of Merrifield (2). Introduction of the dimethylaminonapthalenesulfonyl (dansyl) group was accomplished by deprotection of the BOC-protected amino group of residue 1 with 25% TFA/CH_2Cl_2, neutralization by 10% diisopropylethylamine/CH_2Cl_2, and reaction of dansyl chloride in CH_2Cl_2/DIEA (2:1). The resulting derivative was cleaved from the resin by the action of anhydrous HF and the product extracted with 50% acetic acid/water. The crude peptide was purified by Sephadex G-25 chromatography followed by CM-Sepharose. The correct peak of synthetic-α-DNS-thr^8-(1–16) was selected and the concentration determined by quantitative amino acid analysis.

Fluorescence spectra were recorded at ambient temperature using an Aminco-Bowman Spectrophotofluorometer. After obtaining an excita-tion spectra of the peptide alone, increments of pepsin were added and the spectra recorded as a function of time. The reciprocal of the observed intensities at 15 s and 30 min are plotted versus the reciprocal of the pepsin concentration and the resulting linear plots were extrapolated to infinite concentration to provide the maximum intensity for the complex. These

[1]C. Smith, G. Seegan, R. C. Siegel, V. N. Schumaker, and R. E. Cathou. Manuscript in preparation.