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.

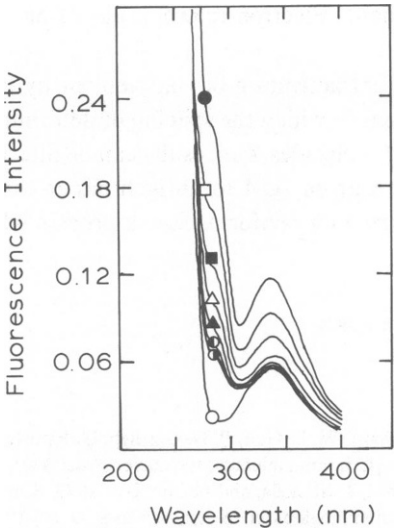

Figure 1 Excitation spectra recorded by observing emission at 525 nm of a solution of α-DNS-Thr^{8}-(1–16) peptide alone (○) and mixed with an equivalent amount of porcine pepsin at pH 5.5. The curves are obtained at 15 s (●), 2 min (□), 4 min (■), 6 min (△), 8 min (▲), 10 min (◐), 20 min, and 30 min (◑).

values were employed to calculate distances from the following relationship:

$$r = \left(\frac{1}{E} - 1\right)^{1/6} R_0,$$

where r is the distance of separation of the interacting chromophores, R_0 is the distance for 50% efficiency of transfer, and E is calculated from ratios of absorbance and fluorescence at the donor and acceptor wavelengths (3).

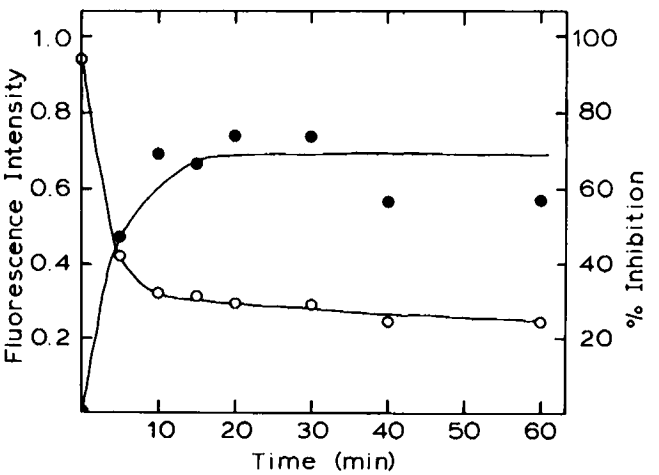

Figure 2 A plot of the fluorescence intensity at 525 nm with excitation at 290 nm vs. time (○) and the percent inhibition of the pepsin measured by aliquot from the same solution (●).

RESULTS AND DISCUSSION

When α-DNS-Thr^8-(1–16) was mixed with pepsin, a very rapid reaction ($t_{1/2} < 3$ s) led to a species with greatly enhanced dansyl fluorescence arising from an additional excitation peak at 290 nm. This may be attributed to energy transfer from Trp residues of pepsin. Since R_0 is known to be 22 Å for the Trp-dansyl pair, we can calculate the distance between the two chromophores of 20.5 Å for this initial complex.

This initial complex is short-lived, however, since the fluorescence decays to a second value with a half life of 2 min. The final level of fluorescence enhancement may be used to calculate a new distance of 31.7 Å. This shift in position of 11.2 Å is of profound significance since the appearance of inhibition of pepsin activity occurs with the same time dependence as the fluorescence decay as shown in Fig. 2.

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STUDIES OF FUNCTIONALLY IMPORTANT STRUCTURAL FLEXIBILITY OF THIOSULFATE SULFURTRANSFERASE

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The enzyme thiosulfate sulfurtransferase (TST; rhodanese; EC 2.8.1.1) catalyzes the transfer of the outer sulfur of thiosulfate ($S_2O_3^-$) to a variety of nucleophilic acceptors such as cyanide (CN^-). During the course of this reaction the enzyme cycles through two stable isolatable catalytic intermediates: the free enzyme, (E), and the sulfur substituted enzyme, (ES). The study of TST is of interest because it displays a number of nonartifactual discrepancies between the x-ray structure (1) and numerous solution studies. The x-ray structure shows that the enzyme is a single polypeptide chain that is folded into two distinct domains with the active site in the interdomain region. Addition of CN^- to crystals of ES is reported not to produce the large conformational changes that would be expected from solution studies (2, 3). In addition, the active site sulfhydryl group has characteristic reactivities in solution (4, 5) that are not possible based on the crystal structure of the ES form. It appears that fluctuations in the structure of TST are in part responsible for these observations, and thus this enzyme will be valuable in developing diagnostics and techniques for the recognition and study of flexibility and domain interactions in proteins.

In the present studies various solution and fluorometric approaches have been used to examine structural fluctuations in TST. These include studies of the solubilities of crystals of