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Interaction of the Fluorescence-Labeled Secretory Component with Human Polymeric Immunoglobulins†

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ABSTRACT: The secretory component (SC) isolated from human milk was labeled with 2 mol of the fluorescent thiol reagent *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide (DACM) per mol of SC through the reactive disulfide bond of SC. The binding of the labeled SC to polymeric immunoglobulins was examined by gel filtration by measuring the fluorescence of DACM at 478 nm. The labeled SC was bound to immunoglobulin M (IgM) and its $(Fc)_{5\mu}$ fragment and to dimeric immunoglobulin A (IgA). When the labeled SC was bound to IgM or the $(Fc)_{5\mu}$ fragment, the fluorescence of DACM increased about 30%. By use of this fluorescence change, quantitative studies were made on the equilibrium and kinetics of the reversible interactions of the labeled SC with two IgM proteins and their $(Fc)_{5\mu}$ fragments at pH 7.0 and 25 °C. All the IgM proteins and their $(Fc)_{5\mu}$ fragments had one binding site per mole of polymers. The affinity constant (6×10^8 M^{-1}), the association rate constant (7×10^7 M^{-1} min^{-1}), and the dissociation rate constant (0.1 min^{-1}) of one IgM were different from those of the other IgM (1.7×10^9 M^{-1}, 1.0×10^8 M^{-1} min^{-1}, and 0.06 min^{-1}, respectively). However, the values for the $(Fc)_{5\mu}$ fragments of the two proteins were the same (1.9×10^9 M^{-1}, 1.1×10^8 M^{-1} min^{-1}, and 0.06 min^{-1}, respectively) and were very similar to those of the IgM with the higher affinity constant. These results suggest that, although SC is bound to Fc regions of the IgM molecule, Fab' regions influence the interaction so as to weaken the affinity and the extent of this influence differs in different IgM proteins.

The secretory component (SC)[1] is a glycoprotein that is synthesized by a variety of glandular epithelial cells (Lamm, 1976; Cunningham-Rundles, 1978) and hepatocytes (Zvenbergen et al., 1980). It is found in external secretions associated with polymeric IgA and IgM and also in the free form. Biochemical and cytochemical studies (Cunningham-Rundles, 1978; Brandtzaeg, 1981; Nagura et al., 1979; Takahasi et al., 1982) have shown that SC functions as a receptor in the transcellular transport of polymeric immunoglobulins across epithelial cells and hepatocytes. Subsequently, SC has been found to be synthesized as a larger precursor transmembrane protein which undergoes intracellular processing by cleavage of SC from the precursor during transcellular transport (Mostov et al., 1980; Kühn & Kraehenbuhl, 1981; Mostov & Blobel, 1982). The finding of the precursor of SC has provided a basis for its function as a receptor. More recently, Mostov et al. (1984) have reported the complete primary structure of the precursor of rabbit SC and proposed that SC consists of multiple immunoglobulin-like domains.

The association of polymeric immunoglobulins with the receptor SC molecule is essential for their transport, and there have been many studies of the interaction of SC with polymeric IgA and IgM [reviewed by Brandtzaeg (1981)]. Results have shown that while the interchain disulfide bonds are important for stabilizing the human SC-IgA complex, the specific noncovalent interactions between SC and the Fc regions of J chain containing polymeric IgA and IgM, with an affinity constant of about 10^8-10^9 M^{-1}, reflect the potential receptor

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[1] Abbreviations: SC, secretory component; IgM, immunoglobulin M; IgA, immunoglobulin A; DACM, *N*-[7-(dimethylamino)-4-methylcoumarinyl]maleimide; DACM-SC, SC in which one disulfide bond is reduced and is coupled with 2 mol of DACM; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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function of SC. However, there remain many uncertainties as to the mechanism of the interaction in terms of the exact protein structure. From the homology in sequence between SC and immunoglobulin domains, Mostov et al. (1984) have suggested that this interaction may resemble that of the immunoglobulin domains with each other.

To examine the mechanism of the interaction in detail, we prepared fluorescence-labeled SC. Free SC isolated from milk has no free thiol groups, but Cunningham-Rundles & Lamm (1975) found that it contains a highly reactive disulfide bond. This disulfide bond has been proposed to be rearranged to yield interchain disulfide bonds with dimeric IgA and to stabilize the SC–IgA complex. To label SC specifically, we took advantage of this reactive disulfide bond; namely, we reduced the reactive disulfide bond and allowed the two resulting thiol groups to react with a thiol-specific fluorescent reagent. Since the label was strongly fluorescent, the binding of the labeled SC to various polymeric immunoglobulins was easily detectable. From the change in fluorescence of the labeled SC on binding, we measured the affinity constants and the time courses of the reversible interactions of the labeled SC with IgM proteins and their $(Fc)_{5\mu}$ fragments. We measured the affinity constant of labeled SC with dimeric IgA protein for comparison with those of the IgM proteins. The results with IgM proteins and their $(Fc)_{5\mu}$ fragments suggested a role of the Fab′ region of the IgM molecule in its interaction with SC.

Materials and Methods

Reagents. DACM was obtained from Teika Seiyaku Co. Trypsin was from Sigma Chemical Co. Dithiothreitol, iodoacetamide, and 2-mercaptoethanol were from Nakarai Chemicals Co. Sephadex and Sepharose were from Pharmacia Fine Chemicals, and DEAE-cellulose-52 and CM-cellulose-52 were from Whatman Biochemicals Ltd. Goat anti-human SC serum and rabbit anti-human lactoferrin serum were from Miles-Yeda Ltd.

Secretory Component. SC was purified from human colostrum as described by Klingmüller & Hilschmann (1979) except that a Sephadex G-200 column (6 × 120 cm) was used instead of a Sephadex G-100 column for gel filtration of the whey. Purified SC gave a single band on SDS–polyacrylamide gel electrophoresis in the presence and absence of 2-mercaptoethanol. A single component was also observed on immunoelectrophoresis of the purified SC using antiserum to SC. No precipitin line was formed when antiserum to lactoferrin was used. The circular dichroism spectrum of the purified SC was measured from 320 to 205 nm with a Jasco J-40 spectropolarimeter. The spectrum was very similar to that reported by Björk & Lindh (1974).

SC in which one disulfide bond was reduced and combined with 2 mol of DACM (DACM-SC) was prepared as follows. About 0.3% (w/v) SC in 0.1 M Tris-HCl buffer, pH 8.0, was allowed to react with 1 mM dithiothreitol for 10 min at 25 °C. The reaction was stopped by lowering the pH to 5 with an appropriate amount of 2 N acetic acid, and then the partially reduced SC was separated from the residual reagents on a column of Sephadex G-25 equilibrated with degassed 0.2 M NaCl solution containing 1 mM EDTA. When the partially reduced SC was titrated with DTNB, its thiol content was always between 1.8 and 2.0. The partially reduced SC was allowed to react with DACM at 2-fold molar excess over the thiol groups of SC in 50 mM sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl for 20 min at 25 °C. Then the labeled SC was separated from residual reagents on a column of Sephadex G-25 equilibrated with 10 mM Tris-HCl buffer, pH 8.0, containing 0.2 M NaCl. The adduct of DACM with thiol is not stable, and its succinimide ring gradually opens to form the corresponding succinamic acid (Yamamoto et al., 1977). To allow transformation of the adduct to a stable form, the solution was kept at pH 8.0 and 4 °C for 3 days before use. The amount of DACM that had reacted was determined from the absorbance at 380 nm by using a molar extinction coefficient of 19 800 M^{-1} cm^{-1} (Yamamoto et al., 1977). In calculation of the concentration of SC, the absorbance of combined DACM at 280 nm was assumed to be the same as that of free DACM. The contribution of DACM to the absorbance of DACM-SC at 280 nm was always small (about 8% of the total absorbance).

IgM. Pentameric IgM proteins were prepared from the sera of two myelomas [human Mi(κ) and Oda(κ)]. The IgM proteins were precipitated by dialysis of the sera against distilled water. The precipitate was dissolved in 20 mM Tris-HCl buffer, pH 8.0, containing 0.2 M NaCl and chromatographed on a column (3.5 × 110 cm) of Sepharose 4B equilibrated with the same buffer. The chromatography was repeated several times. Analysis of the purified IgM proteins by SDS–polyacrylamide gel electrophoresis on a 3.5% gel in the absence of a reducing reagent showed that the content of the pentameric IgM was more than 95%.

$(Fc)_{5\mu}$ Fragment. $(Fc)_{5\mu}$ fragments were isolated from two IgM proteins by a modification of the method of Plaut & Tomasi (1970). The proteins were digested with trypsin for 30 min at 63 °C at a substrate to enzyme ratio of 25:1 (w/w) in 20 mM Tris-HCl buffer, pH 8.0, containing 0.2 M NaCl. The digestion was stopped by adding soybean trypsin inhibitor at an inhibitor to enzyme ratio of 1:1 (w/w). The digest was applied to a Sephadex G-200 column (3.5 × 110 cm) equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and subjected to ion-exchange chromatography on a DEAE-cellulose column equilibrated with the same buffer. One peak was eluted with a linear gradient of 0–0.3 M NaCl in the same buffer. Fractions in the peak were analyzed by SDS–polyacrylamide gel electrophoresis on a 3.5% gel in the absence of a reducing reagent, and those in which the proportion of the major band with an apparent molecular weight of about 350 000 was more than 90% were stored. After SDS–polyacrylamide gel electrophoresis on a 5.6% gel in the presence of 2-mercaptoethanol, the purified $(Fc)_{5\mu}$ fragment gave a major band with an apparent molecular weight of about 70 000 and no band of intact μ chain.

Dimeric IgA. Dimeric IgA was prepared from the serum of a myeloma [human Mo(κ)]. The precipitate of the serum with 50% saturation of ammonium sulfate was dissolved in and dialyzed against 10 mM Tris-HCl buffer, pH 8.0, and subjected to ion-exchange chromatography on a DEAE-cellulose column. On elution with a linear gradient of 0–0.2 M NaCl in the same buffer, one major peak with a shoulder on the side of lower NaCl concentration was obtained. The predominant form in the peak was dimeric IgA, while fractions in the shoulder contained monomeric IgA. The fractions in the major peak were pooled and chromatographed several times on a column (3 × 110 cm) of Sepharose 6B equilibrated with 20 mM Tris-HCl buffer, pH 8.0, containing 0.2 M NaCl. The purified dimeric IgA was found to contain 5% of larger polymers by SDS–polyacrylamide gel electrophoresis on a 3.5% gel in the absence of a reducing reagent.

For the competitive binding test, dimeric IgA was treated with iodoacetamide in 0.1 M Tris-HCl buffer, pH 8, containing 0.2 M NaCl for about 10 h at 4 °C, and then the IgA(Mo) was separated from the residual reagent on a column of

Sephadex G-25 equilibrated with 20 mM sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl.

Secretory IgA. Secretory IgA was prepared from human colostrum. Whey was applied to a Sephadex G-200 column, and the first peak eluted containing secretory IgA was purified further as described by Iscaki et al. (1978). The purified protein contained about 10% of larger polymers, as judged by SDS–polyacrylamide gel electrophoresis.

DACM Attached to 2-Mercaptoethanol. DACM (20 μM) was treated with 2-mercaptoethanol (2 mM) in 20 mM sodium phosphate buffer, pH 7, overnight at 40 °C. Then the pH was raised to 8 by adding an appropriate amount of 1 M Tris base, and the solution was kept for 3 days at 4 °C to allow transformation of the adduct to a stable form.

Analytical Gel Filtration. Binding of DACM-SC to various immunoglobulins was measured by Sephadex G-200 (1.6 × 97 cm) chromatography. Mixtures of DACM-SC and various immunoglobulins in 20 mM sodium phosphate buffer, pH 7.0, containing 0.2 M NaCl were incubated overnight at 25 °C. The concentration of DACM-SC was usually 1 μM. After incubation, 2.5-mL volumes of the mixtures were applied to columns equilibrated with the same buffer. The columns were thermostatically regulated at 25 °C with circulating water. Fractions of 2.4 mL were collected at a flow rate of 6 mL/h, and the fluorescence of DACM at 478 nm and the absorbance at 280 nm were measured.

Fluorescence Measurements. Fluorescence was measured with a Hitachi fluorescence spectrophotometer (Model 650-60). The temperature was kept at 25 °C by using a thermostatically controlled cell holder. The fluorescence of DACM was measured by using 383-nm light for excitation. For titrations of DACM-SC with various immunoglobulins, 1-mL volumes of a fixed concentration of DACM-SC were mixed with 1 mL of various concentrations of immunoglobulins in a cell, and after 30 min at 25 °C, the fluorescence was measured. Examination of the binding kinetics showed that 30 min was long enough for the system to reach equilibrium. For kinetic measurements, 1 mL of DACM-SC solution at 25 °C was added to 1 mL of immunoglobulin solution at 25 °C in a cell, and the time-dependent change in fluorescence at 478 nm was measured. The dead time of measurements was 15 s. Analyses of the titrations at low concentrations of DACM-SC and of the binding kinetics were carried out by fitting the curve to a theoretical model using a nonlinear least-squares program based on the Marquardt algorithm (Marquardt, 1963) with a Hewlett-Packard Model 9826A computer.

Nature of Interaction between DACM-SC and Immunoglobulins. For examination of the nature of the bonds formed between DACM-SC and immunoglobulins, the complex eluted from the Sephadex G-200 column was subjected to SDS–polyacrylamide gel electrophoresis on a 3.5% gel in the absence of a reducing reagent. Covalently bound DACM-SC should migrate slowly with polymeric immunoglobulins, whereas noncovalently bound DACM-SC should migrate fast. After electrophoresis, the gel was sectioned under ultraviolet light to separate free DACM-SC from DACM-SC bound to immunoglobulins. DACM-SC was extracted from the gel, and its fluorescence intensity was measured as described by Yamamoto et al. (1977).

Titration of Thiol Groups with DTNB. The thiol contents of intact SC and the partially reduced SC were measured by titration with DTNB. To 2.5 mL of SC solution was added 0.5 mL of freshly prepared 5 mM DTNB solution in 0.5 M Tris-HCl buffer, pH 8.2, containing 4 M guanidine hydrochloride and 1 mM EDTA, and its absorbance at 412 nm was measured against a freshly prepared reagent blank. The final pH of the reaction mixture was 8.0. The molar extinction coefficient of reduced DTNB was assumed to be 13 600 M^{-1} cm^{-1} at 412 nm (Gething & Davidson, 1972). All the buffers used for titration of thiol groups were degassed.

Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis in the presence of SDS was carried out essentially as described by Weber & Osborn (1969). When the electrophoresis was carried out in the absence of a reducing reagent, the protein solutions used were treated with 5 mM iodoacetamide overnight at pH 7–8 before addition of SDS.

The presence of the J chain was examined by polyacrylamide gel electrophoresis at pH 9.4 on a 5.6% gel in the presence of 10 M urea essentially by the method of Reisfeld & Small (1966). All the immunoglobulins used, two IgM proteins and their $(Fc)_{5\mu}$ fragments, dimeric IgA(Mo), and secretory IgA, were found to contain the J chain.

Protein Concentration. Protein concentration was measured spectrophotometrically. The absorption coefficient at 280 nm for 1% (w/v) solutions of proteins in 1-cm cells ($A^{1\%}_{1cm}$) was determined as described by Lodola et al. (1978), except that the concentration of a protein solution of known absorbance was determined from the refractive index of the solution by using a Yanaco R-202 refractometer. The value of $A^{1\%}_{1cm}$ at 280 nm was 13.1 for IgM(Mi), 11.2 for IgM(Oda), 11.1 for dimeric IgA(Mo), and 10.1 for the $(Fc)_{5\mu}$ fragment; that for SC was assumed to be 12.1 (Lindh & Björk, 1974). Values for molecular weights were taken as 900 000 for IgM (Dorrington & Tanford, 1970), 342 000 for the $(Fc)_{5\mu}$ fragment (Plaut & Tomasi, 1970), 360 000 for dimeric IgA, and 75 000 for SC (Lindh & Björk, 1974).

Results

Preparation of DACM-SC. Titration with DTNB showed that intact SC isolated from human milk has no free thiol groups. When SC was partially reduced by 1 mM dithiothreitol at pH 8.0 and 30 °C for 10 min, 1.8–2.0 mol of thiol groups per mol of SC was detected. The value increased slightly with increase in the reduction time and was 2.5 mol of thiol groups after reduction for 1 h. These results are consistent with the report of Cunningham-Rundles & Lamm (1975) that one disulfide bond of SC is especially sensitive to reduction. Partially reduced SC obtained by reduction for 10 min was then treated with the fluorescent thiol reagent DACM as described under Materials and Methods. The number of DACM molecules introduced per mole of SC was consistently 1.9–2.2. The corrected fluorescence spectrum of the labeled SC (DACM-SC) had a maximum at 483 nm on excitation at 383 nm (Figure 3).

Binding Measured by Gel Filtration. For the purpose of this work, it was important to demonstrate that DACM-SC showed an affinity of binding similar to that of intact SC to polymeric immunoglobulins. Therefore, we first examined the binding of DACM-SC to polymeric immunoglobulins. Figure 1 shows the gel filtration patterns on a Sephadex G-200 column of DACM-SC and mixtures of DACM-SC and various polymeric immunoglobulins. Fluorescence at 478 nm represents the elution of DACM-SC. On the other hand, absorption at 280 nm mostly represents the elution of immunoglobulins because the absorption of DACM-SC under the experimental conditions was very small, as can be seen from Figure 1a.

In the presence of a 3-fold molar excess of IgM(Mi), most of the DACM-SC was eluted much faster (Figure 1b) than DACM-SC alone, and the elution position of DACM-SC

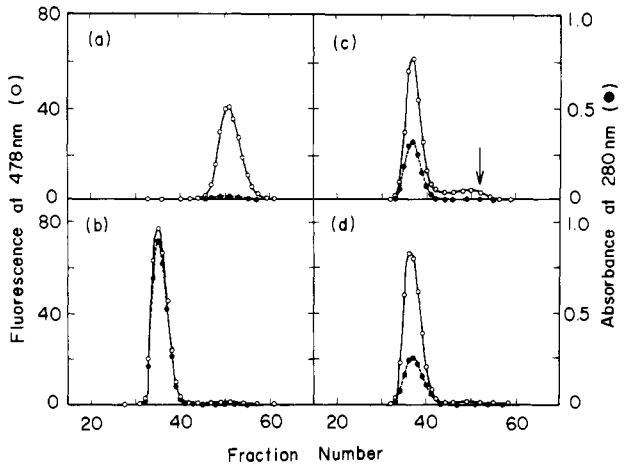

FIGURE 1: Gel filtration of mixtures of various immunoglobulins and DACM-SC at pH 7.0 and 25 °C. Volumes of 2.5 mL of solution contained DACM-SC (1 μM) alone (a) or with various immunoglobulins (3 μM): (b) IgM(Mi); (c) dimeric IgA(Mo); (d) $(Fc)_{5\mu}$ fragment of IgM(Mi). The solutions were applied to a Sephadex G-200 column (1.6 × 97 cm). Fractions of 2.4 mL were collected, and their fluorescence at 478 nm (○) and absorbance at 280 nm (●) were measured. The arrow in (c) indicates the elution position of free SC.

coincided with that of IgM(Mi), indicating that the DACM-SC was bound completely to IgM(Mi). The fluorescence intensity of DACM-SC eluted with IgM(Mi) was higher than that of DACM-SC alone, due to increase in fluorescence on binding, as shown later in Figure 3. The other IgM protein, IgM(Oda), was also found to bind DACM-SC completely when examined under the same conditions (not shown). Figure 1c shows the gel filtration pattern of a mixture of DACM-SC and a 3-fold excess of dimeric IgA(Mo). The elution profile showed substantial binding of DACM-SC to dimeric IgA(Mo). However, the binding was not complete, and the fluorescence in the main peak represented 88% of the total. The second fluorescence peak containing 12% of the total was eluted slightly faster than free SC. These results indicate that the affinity of DACM-SC for dimeric IgA(Mo) was lower than that for IgM and that some dissociation of the complex occurred during gel filtration. We also examined the binding of DACM-SC to the $(Fc)_{5\mu}$ fragment. Although the $(Fc)_{5\mu}$ fragment is only one-third the size of the IgM molecule, as is apparent from the elution profile detected by the absorbance at 280 nm, the $(Fc)_{5\mu}$ fragment of IgM(Mi) bound DACM-SC completely. The $(Fc)_{5\mu}$ fragment obtained from IgM(Oda) also bound DACM-SC tightly. On the contrary, chromatography under similar conditions showed that a negligible amount of DACM-SC was bound to secretory IgA prepared from colostrum, which already bound SC (not shown).

For determination of whether the binding was covalent, the complex eluted from the column was subjected to SDS–polyacrylamide gel electrophoresis on a 3.5% gel in the absence of a reducing reagent. The proportions of noncovalently bound SC and covalently bound SC were determined from the distribution of the fluorescence of DACM as described under Materials and Methods. In all the complexes described above, binding was exclusively noncovalent.

We then examined the effect of introduction of DACM into SC by a competitive binding test. First, as a control, a mixture of IgM and a 2-fold molar excess of DACM-SC were subjected to gel filtration (Figure 2a). DACM-SC was eluted in the positions of the complex and of free SC, 51.4% of the fluorescence intensity being in the position of the complex and 48.6% in that of free DACM-SC. By correction of the value for the fluorescence intensity of the complex by dividing it by

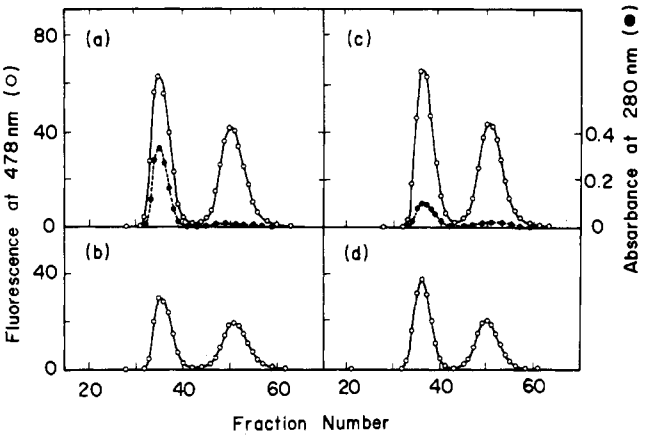

FIGURE 2: Competitive bindings of DACM-SC and intact SC to IgM(Mi) and its $(Fc)_{5\mu}$ fragment at pH 7.0 and 25 °C. Volumes of 2.5 mL of solutions contained (a) DACM-SC (2 μM) and IgM(Mi) (1 μM), (b) DACM-SC (1 μM), intact SC (1 μM), and IgM(Mi) (1 μM), (c) DACM-SC (2 μM) and $(Fc)_{5\mu}$ fragment (1 μM), and (d) DACM-SC (1 μM), intact SC (1 μM), and $(Fc)_{5\mu}$ fragment (1 μM). The solutions were applied to a Sephadex G-200 column (1.6 × 97 cm). Fractions of 2.4 mL were collected, and their fluorescence at 478 nm (○) and absorbance at 280 nm (●) were measured.

Table I: Stoichiometries of the Interactions of DACM-SC with Polymeric Immunoglobulins Determined by Fluorescence Titration at pH 7.0 and 25 °C

protein	stoichiometry[a] (mol of DACM-SC/mol of polymer)	fluorescence increase by the interaction (%)[b]
IgM(Mi)	0.83 (0.88)	35
IgM(Oda)	0.94 (0.87)	34
$(Fc)_{5\mu}$ of IgM(Mi)	1.03 (0.84)	32
$(Fc)_{5\mu}$ of IgM(Oda)	1.03 (0.92)	30
IgA(Mo) dimer	(0.80)	17[c]

[a] Values in parentheses were determined by gel filtration (see the text for details). [b] Percentage increase over the fluorescence intensity of free DACM-SC at 478 nm. [c] Value determined by fluorescence titration at low DACM-SC concentration.

a factor of 1.35 (see Table I), it was calculated that 44% of DACM-SC was bound to IgM(Mi). This means that 0.88 mol of DACM-SC was bound per mol of IgM, indicating a stoichiometry of binding of about 1:1. Next we subjected a similar mixture in which half the DACM-SC was replaced by intact SC to gel filtration (Figure 2a). If the binding properties of intact SC and DACM-SC are the same, we could expect an elution profile in which the fluorescence intensities of the respective peaks were half of those in the control profile (Figure 2a). If the affinity of intact SC for IgM is stronger than that of DACM-SC, the fluorescence intensity of the first peak should be less than half, and if the affinity of DACM-SC for IgM is stronger, it should be more than half that of the control. As shown in Figure 2b, the shape of the elution profile was similar to that of the control, and the heights of peaks were about half those of the control. From the fluorescence intensities of the peaks corrected for the fluorescence increase due to complex formation, it was calculated that 43% of DACM-SC was eluted as a complex. This value was very similar to the proportion of complex (44%) in the control gel filtration. This indicated that the binding properties of DACM-SC and intact SC to IgM(Mi) were very similar.

Figure 2c,d shows the results of similar experiments with the $(Fc)_{5\mu}$ fragment of IgM(Mi). When a mixture of the $(Fc)_{5\mu}$ fragment and a 2-fold molar excess of DACM-SC was chromatographed, 42% of DACM-SC was eluted as a complex with the $(Fc)_{5\mu}$ fragment. This means that 0.84 mol of

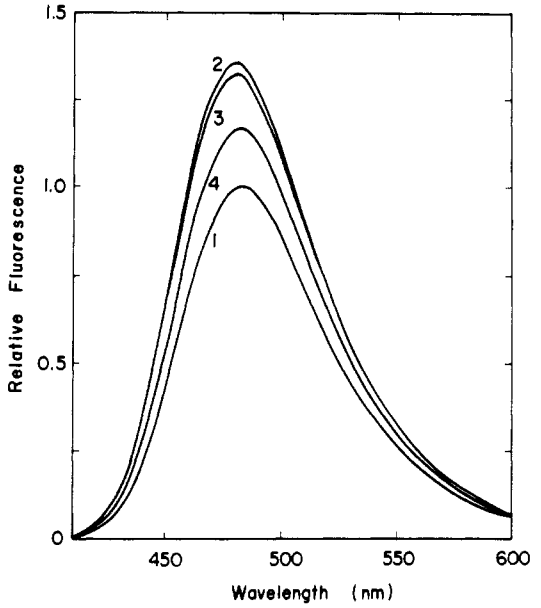

FIGURE 3: Corrected fluorescence spectra of DACM-SC (1) and DACM-SC bound to IgM(Mi) (2), to its $(Fc)_{5\mu}$ fragment (3), and to dimeric IgA(Mo) (4) at pH 7.0 and 25 °C. The final concentrations of DACM-SC, IgM(Mi), $(Fc)_{5\mu}$ fragment, and dimeric IgA(Mo) were 0.2, 0.4, 0.4, and 0.7 μM, respectively. Excitation was at 383 nm.

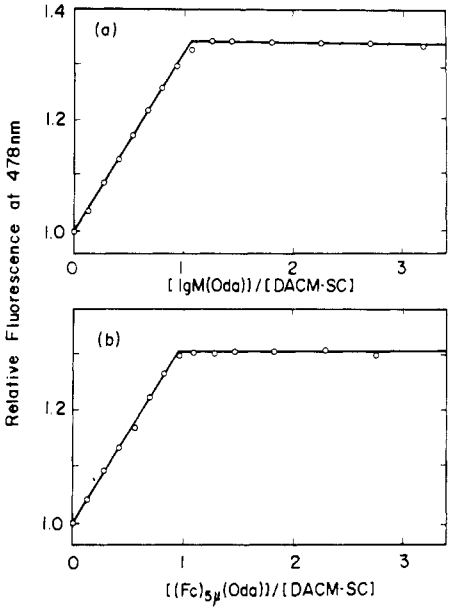

FIGURE 4: Fluorescence titration of DACM-SC with IgM(Oda) (a) and with its $(Fc)_{5\mu}$ fragment (b) at higher protein concentrations at pH 7.0 and 25 °C. The concentration of DACM-SC was 0.3 μM. Excitation was at 383 nm, and the fluorescence at 478 nm was measured.

DACM-SC was bound per mol of $(Fc)_{5\mu}$ fragment (Table I). When half the DACM-SC was replaced by intact SC, the shape of the elution profile was very similar to that of the control, and 44% of DACM-SC was eluted as a complex. This showed that the affinity of DACM-SC for the $(Fc)_{5\mu}$ fragment of IgM(Mi) was also very similar to that of intact SC.

Similar competitive binding experiments were carried out on other polymeric immunoglobulins. The results on IgM(Oda) and its $(Fc)_{5\mu}$ fragment were very similar to that on IgM(Mi) (Table I). In the case of dimeric IgA(Mo), we used IgA protein that had been treated with iodoacetamide to alkylate its free thiol groups and thus prevent formation of disulfide bonds between intact SC and dimeric IgA molecule. When a mixture of dimeric IgA(Mo) and a 2-fold molar excess of DACM-SC was chromatographed, the proportion of DACM-SC eluted as a complex was 40%. On the other hand, when half the DACM-SC was replaced by intact SC, the proportion was 30%, indicating a lower affinity of DACM-SC. Although the affinity of DACM-SC for dimeric IgA was a little lower than that of intact SC, there were no differences between the affinities of the two for IgM proteins and $(Fc)_{5\mu}$ fragments. Therefore, we conclude that DACM-SC could be used for study of the interaction of SC with polymeric immunoglobulins.

Fluorescence Spectra. Figure 3 shows the corrected fluorescence spectra of DACM-SC in free and bound forms to various polymeric immunoglobulins. The spectra of DACM-SC bound to IgM(Oda) and its $(Fc)_{5\mu}$ fragment were very similar to those of DACM-SC bound to IgM(Mi) and its $(Fc)_{5\mu}$ fragment, respectively. The fluorescence spectrum of free DACM-SC had a maximum at 483 nm, which was at only 2 nm shorter wavelength than the maximum (485 nm) for DACM attached to 2-mercaptoethanol. When DACM-SC was bound to polymeric immunoglobulins, the fluorescence intensity increased. The extent of increase depended on the protein, the increase being large (30–35%) for complexes with IgM proteins and their $(Fc)_{5\mu}$ fragments but small (about 15%) for the complex with dimeric IgA. The maximum wavelength shifted to a shorter wavelength on complex formation, but the shift was only about 2 nm for all the complexes.

Table II: Affinity and Rate Constants of the Interaction of DACM-SC with Polymeric Immunoglobulins at pH 7.0 and 25 °C[a]

protein	$K_a \times 10^{-8}$ (M^{-1})	$k_1 \times 10^{-7}$ (M^{-1} min^{-1})	$k_{-1} \times 10^{2}$ (min^{-1})
IgM(Mi)	6.3 ± 1.3 (4)	6.8 ± 1.0 (14)	10.7 ± 0.2 (14)
IgM(Oda)	17.1 ± 1.7 (3)	9.9 ± 1.0 (6)	5.8 ± 0.6 (6)
$(Fc)_{5\mu}$ of IgM(Mi)	19.7 ± 1.7 (2)	10.7 ± 1.8 (9)	5.4 ± 0.9 (9)
$(Fc)_{5\mu}$ of IgM(Oda)	17.7 ± 0.3 (2)	11.8 ± 0.6 (7)	6.7 ± 0.3 (7)
IgA(Mo) dimer	0.26 ± 0.01 (2)		

[a] Results are expressed as mean values ± SD. Numbers in parentheses indicate number of experiments.

To estimate the significance of the fluorescence changes, we measured the fluorescence spectra of DACM attached to 2-mercaptoethanol in a series of water–ethanol mixtures. With an increase in the proportion of ethanol, the maximum wavelength shifted to shorter wavelengths, and the fluorescence intensity increased. In 100% ethanol, the maximum was at 462 nm, and the fluorescence intensity was 270% of that in water. These changes on decrease in the polarity of solvent were much greater than those observed for the interaction of DACM-SC with polymeric immunoglobulins.

Fluorescence Titration. We studied the interaction on the basis of the fluorescence increase on complex formation. Figure 4a shows results on the titration of DACM-SC with IgM(Oda) to determine the stoichiometry of binding. The concentration of DACM-SC (0.3 μM) was sufficiently high to bind most of the IgM added below the saturation according to the affinity constant described below (Table II). A plot of the fluorescence intensity at 478 nm against the concentration of IgM gave a curve with a sharp break. From the break point, the stoichiometry of binding was calculated to be 0.94 mol of DACM-SC per mol of IgM (Table I). The fluorescence increased by 34% on complex formation. Figure 4b shows the titration of DACM-SC with the $(Fc)_{5\mu}$ fragment of IgM(Oda). The stoichiometry was 1.03 mol of DACM-SC per mol of $(Fc)_{5\mu}$ fragment. The fluorescence increase by complex formation was 30%. Titrations were also carried out with IgM(Mi) and its $(Fc)_{5\mu}$ fragment, and the results are

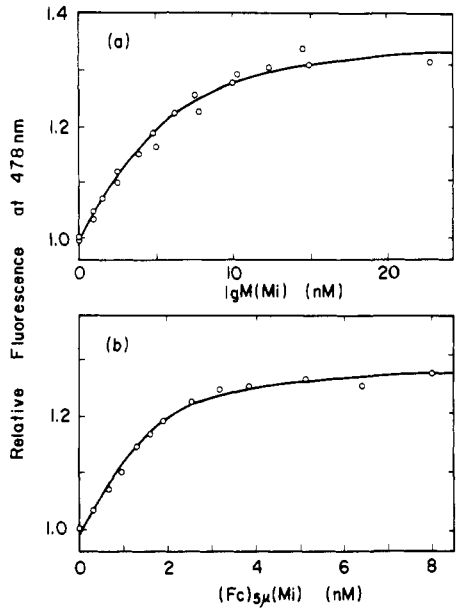

FIGURE 5: Fluorescence titrations of DACM-SC with IgM(Mi) (a) and with its $(Fc)_{5\mu}$ fragment (b) at lower protein concentrations at pH 7.0 and 25 °C. The concentrations of DACM-SC were (a) 5 nM and (b) 1.5 nM. Excitation was at 383 nm, and the fluorescence at 478 nm was measured. The solid lines are theoretical curves calculated according to eq 5 (see the text) by using the following values: (a) $K_a = 5.14 \times 10^8$ M^{-1}, $F_0 = 0.99$, and $F_{max} = 1.36$; (b) $K_a = 2.08 \times 10^9$ M^{-1}, $F_0 = 0.99$, and $F_{max} = 1.30$.

summarized in Table I. For all the proteins examined, the stoichiometry of binding determined by the fluorescence titration was very near to 1 mol of DACM-SC per mol of polymeric immunoglobulin, and the values were slightly higher than those determined by gel filtration.

Because the stoichiometry of binding obtained by titrations was very close to 1:1, we assumed a reversible binding equilibrium of DACM-SC and polymeric immunoglobulins described by the following mechanism:

$$\text{DACM-SC} + \text{pIg} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{DACM-SC–pIg} \qquad (1)$$

where pIg and DACM-SC–pIg represent the polymeric immunoglobulin and the complex of DACM-SC and polymeric immunoglobulin, respectively, and k_1 and k_{-1} represent the association rate constant and the dissociation rate constant, respectively. The affinity constant, K_a, is defined as follows:

$$K_a = \frac{k_1}{k_{-1}} = \frac{[\text{DACM-SC–pIg}]}{[\text{DACM-SC}][\text{pIg}]} \qquad (2)$$

where [DACM-SC], [pIg], and [DACM-SC–pIg] represent the concentrations of free DACM-SC, free polymeric immunoglobulin, and the complex, respectively. The total concentrations of DACM-SC, $[\text{DACM-SC}]_0$, and immunoglobulin, $[\text{pIg}]_0$, are related by

$$[\text{DACM-SC}]_0 = [\text{DACM-SC}] + [\text{DACM-SC–pIg}] \qquad (3)$$

and

$$[\text{pIg}]_0 = [\text{pIg}] + [\text{DACM-SC–pIg}] \qquad (4)$$

To determine the affinity constant, fluorescence titrations of DACM-SC with polymeric immunoglobulins were carried out at low DACM-SC concentrations of about 10^{-9} M. Panels a and b of Figure 5 show the titrations with IgM(Mi) and its $(Fc)_{5\mu}$ fragment, respectively. Titration with the $(Fc)_{5\mu}$ fragment was carried out at a lower concentration of DACM-SC than titration with IgM(Mi) because the affinity for the $(Fc)_{5\mu}$ fragment was stronger. For both proteins, smooth saturation curves that could be used to determine K_a were obtained.

The fluorescence intensity in the presence of polymeric immunoglobulin, $F([\text{pIg}]_0)$, is expressed as

$$F([\text{pIg}]_0) = F_0\frac{[\text{DACM-SC}]}{[\text{DACM-SC}]_0} + F_{max}\frac{[\text{DACM-SC–pIg}]}{[\text{DACM-SC}]_0} \qquad (5)$$

where F_0 and F_{max} represent the relative fluorescence intensities when all the DACM-SC is free and is bound to polymeric immunoglobulins, respectively. From eq 2–4, eq 5 is rewritten as

$$F([\text{pIg}]_0) = F_0 + (F_{max} - F_0)\frac{D - \sqrt{D^2 - 4[\text{DACM-SC}]_0[\text{pIg}]_0}}{2[\text{DACM-SC}]_0} \qquad (6)$$

where

$$D = [\text{DACM-SC}]_0 + [\text{pIg}]_0 + 1/K_a \qquad (7)$$

Although we had determined the ratio of F_{max} to F_0 by titration at high concentrations of DACM-SC (Table I), both F_0 and F_{max} were taken as adjustable parameters. Thus, with eq 6, the values of K_a, F_0, and F_{max} were calculated by nonlinear least-squares analysis (Marquardt, 1963). Analysis of the titration data for IgM(Mi) (Figure 5a) gave values of $K_a = 5.14 \times 10^8$ M^{-1}, $F_0 = 0.99$, and $F_{max} = 1.36$. Analysis of the titration data for the $(Fc)_{5\mu}$ fragment (Figure 5b) gave values of $K_a = 2.08 \times 10^9$ M^{-1}, $F_0 = 0.99$, and $F_{max} = 1.30$. Titrations were also carried out for IgM(Oda) and its $(Fc)_{5\mu}$ fragment and for dimeric IgA(Mo). The values of F_0 and F_{max} for IgM(Oda) and its $(Fc)_{5\mu}$ fragment were consistent with the results obtained by titrations at high concentrations of DACM-SC, and the values of K_a are shown in Table II. With dimeric IgA(Mo), the fluorescence increase by complex formation was determined from the ratio of F_{max} to F_0 to be 17% (Table I). The value of K_a for dimeric IgA(Mo) was 0.26×10^8 M^{-1}. As shown in Table II, the affinity constant was about 3 times larger for the $(Fc)_{5\mu}$ fragment of IgM(Mi) than for IgM(Mi). On the other hand, the affinity constant for the $(Fc)_{5\mu}$ fragment of IgM(Oda) was very similar to that for IgM(Oda). The affinity constants for the two $(Fc)_{5\mu}$ fragments were very similar. The affinity constant for dimeric IgA(Mo) was much smaller than those for the IgM proteins.

Binding Kinetics. The greatest advantage of the fluorescent probe was that it could be used to follow the time course of the interaction directly. Panels a and b of Figure 6 show the time course of binding of DACM-SC to IgM(Mi) and its $(Fc)_{5\mu}$ fragment, respectively. Similar time-dependent changes in fluorescence were observed for the interactions of DACM-SC with IgM(Oda) and its $(Fc)_{5\mu}$ fragment. The concentrations of DACM-SC and immunoglobulins were similar to those used in fluorescence titrations to determine K_a. Under these conditions, the reaction became rapid with increase in the concentration of polymeric immunoglobulin. The fluorescence intensities after equilibration were consistent with those expected from the fluorescence titrations.

When the interaction is described by eq 1, the time course of the binding under the above conditions should be governed by both the association rate constant (k_1) and the dissociation rate constant (k_{-1}). Therefore, these rate constants can be determined by analysis of the time course of the interactions. The fluorescence intensity at time t after mixing the solutions

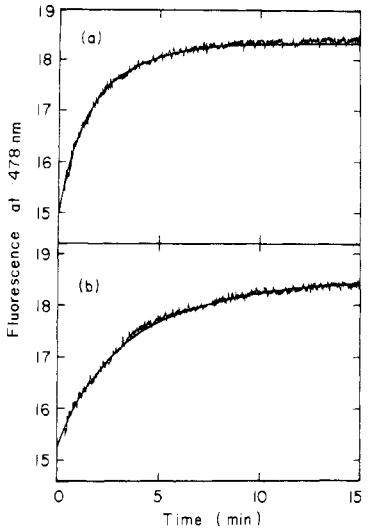

FIGURE 6: Binding kinetics of DACM-SC to IgM(Mi) (a) and to its $(Fc)_{5\mu}$ fragment (b) at pH 7.0 and 25 °C. To 1 mL of solution of IgM or $(Fc)_{5\mu}$ fragment was added 1 mL of solution of DACM-SC, and the fluorescence change at 478 nm was measured. Excitation was at 383 nm. Final conditions: (a) [DACM-SC], 5 nM; [IgM(Mi)], 6.1 nM; (b) [DACM-SC], 1.5 nM; [$(Fc)_{5\mu}$] fragment, 2.6 nM. The noisy curves depict the raw data, while the smooth curves are the theoretical ones calculated according to eq 9 (see the text) by using the following values: (a) $k_1 = 7.4 \times 10^7$ M^{-1} min^{-1}, $k_{-1} = 0.12$ min^{-1}, $F_0 = 14.9$, and $F_{max} = 20.2$; (b) $k_1 = 9.5 \times 10^7$ M^{-1} min^{-1}, $k_{-1} = 0.048$ min^{-1}, $F_0 = 15.2$, and $F_{max} = 19.7$.

of DACM-SC and polymeric immunoglobulin, $F(t)$, is expressed as

$$F(t) = F_0\frac{[\text{DACM-SC}]}{[\text{DACM-SC}]_0} + F_{max}\frac{[\text{DACM-SC-pIg}]}{[\text{DACM-SC}]_0} \quad (8)$$

From eq 2–4, eq 8 can be expressed as

$$F(t) = F_0 + (F_{max} - F_0)\frac{Y}{[\text{DACM-SC}]_0} \quad (9)$$

where

$$Y = \frac{\gamma\beta[1 - \exp(k_1 t\sqrt{Q})]}{2\gamma \exp(k_1 t\sqrt{Q}) - 2\beta} \quad (10)$$

$$Q = D^2 - 4[\text{DACM-SC}]_0[\text{pIg}]_0 \quad (11)$$

$$\beta = -D + \sqrt{Q} \quad (12)$$

and

$$\gamma = -D - \sqrt{Q} \quad (13)$$

In the analysis, we could use the value for K_a obtained by equilibrium measurements. Thus, in eq 9, the values of k_1, F_0, and F_{max} were taken as adjustable parameters and were calculated by nonlinear least-squares analysis (Marquardt, 1963). The value of k_{-1} was then calculated from the relation $k_{-1} = k_1/K_a$. To obtain accurate values, we measured the binding kinetics under various conditions in which the proportion of the complexed DACM-SC after equilibration varied from 30% to 90%. For each immunoglobulin, the respective values were independent of the concentration of the immunoglobulin, and the values of F_0 and F_{max} were consistent with those determined by equilibrium measurements. These findings support eq 1 for the kinetics of the interaction of DACM-SC with IgM proteins and their $(Fc)_{5\mu}$ fragments. The average values of k_1 and k_{-1} are shown in Table II. The values for IgM(Oda) and its $(Fc)_{5\mu}$ fragment and for the $(Fc)_{5\mu}$ fragment of IgM(Mi) were similar, like their affinity constants, and their association rate constant (k_1) and dissociation rate constant (k_{-1}) were about 1×10^8 M^{-1} min^{-1} and 0.06 min^{-1}, respectively. The k_1 of IgM(Mi) (7×10^7 M^{-1} min^{-1}) was a little smaller and its k_{-1} (0.1 min^{-1}) a little larger than the respective values for other proteins.

Discussion

Equilibrium and Kinetics of the Interaction. The interaction of SC with polymeric immunoglobulins is essential for its function. To examine this interaction, we prepared specifically fluorescence-labeled SC. SC isolated from milk contains one highly reactive disulfide bond. This disulfide bond was reduced, and the two resulting thiol groups were allowed to react with the thiol-specific fluorescent reagent DACM. DACM has a high molar extinction coefficient and a high quantum yield when it reacts with protein thiol groups (Yamamoto et al., 1977). It sensitivity is so high that it can be used in studies of this interaction with solutions of as low concentration as 1 nM SC.

To establish the validity of use of DACM-SC, we first measured the binding of DACM-SC to polymeric immunoglobulins by gel filtration. As shown in Figure 1, DACM-SC is bound specifically to polymeric immunoglobulins containing the J chain. Because the cysteine residues that may form interchain disulfide bonds with polymeric immunoglobulins are blocked by DACM, the binding is by noncovalent interaction. Comparison of the affinity of DACM-SC for polymeric immunoglobulins with that of intact SC by competitive binding (Figure 2) showed no difference between the affinities for IgM proteins and their $(Fc)_{5\mu}$ fragments. Although the affinity of DACM-SC for dimeric IgA was weaker than that of intact SC, the difference was small. Thus, the influence of the two introduced DACM groups on the affinity of SC for polymeric immunoglobulins was small, and use of DACM-SC in this study was valid.

The increases in fluorescence accompanying the binding of DACM-SC to IgM and the $(Fc)_{5\mu}$ fragment were large. Therefore, we could carry out quantitative studies on two IgM proteins and their fragments by measuring this fluorescence increase. Because the stoichiometry of interaction was very close to 1:1 for all the polymeric immunoglobulins examined, we analyzed the results assuming simple bimolecular binding equilibrium. The affinity constants of the two IgM proteins were about 10^9 M^{-1}. The affinity constant for the interaction of SC with polymeric immunoglobulin has been reported to be about 10^8 M^{-1} (Brandtzaeg, 1977; Socken & Underdown, 1978). Thus, our values for the constants of IgM proteins are slightly higher than those reported. From the time course of the binding, we estimated the association rate constant (k_1) and the dissociation rate constant (k_{-1}) to be about 10^8 M^{-1} min^{-1} and 0.1 min^{-1}, respectively. From the primary structure of the precursor of rabbit SC, Mostov et al. (1984) proposed that SC consists of multiple immunoglobulin-like domains. They further suggested that the interaction between SC and polymeric immunoglobulins somehow resembles that of the immunoglobulin domains with each other. The interactions between various domains of immunoglobulin have been studied, namely, those of the heavy chain–light chain (Bigelow et al., 1974; Azuma & Hamaguchi, 1975; Friedman et al., 1978; Watt & Voss, 1979), light chain–light chain (Green, 1973; Azuma et al., 1974), and variable fragment–variable fragment (Maeda et al., 1976; Azuma et al., 1978). Large differences were found in the values of the affinity constants and kinetic constants of these interactions. Bigelow et al. (1974) estimated the affinity constant between the heavy and light chains to be

at least 10^{10} M^{-1}, and Friedman et al. (1978) reported that the association rate constant of this interaction was 3.6×10^8 M^{-1} min^{-1}. Maeda et al. (1976) reported an association rate constant of 5.4×10^8 M^{-1} min^{-1} for the variable fragment–variable fragment interaction. The values of the affinity constant (about 10^9 M^{-1}) and association rate constant (about 10^8 M^{-1} min^{-1}) obtained for the interaction of SC with IgM proteins are comparable with these upper values for the immunoglobulin domain–domain interaction. This fact is consistent with the idea that the interaction is similar to that between the immunoglobulin domains and suggests that some immunoglobulin-like domains of the SC molecule interact specifically with a particular domain (or domains) of the polymeric immunoglobulin containing the J chain. As other specific protein–protein interactions, various enzyme–protein inhibitor systems, in which the affinity constant is as high as 10^{10} M^{-1} or more, have been studied extensively. The k_1 values for the interaction of enzymes and inhibitors are relatively similar, ranging from 0.4×10^8 to 5×10^8 M^{-1} min^{-1} (Uehara et al., 1980). The association rate constants for the interactions of DACM-SC with IgM proteins are comparable with those in enzyme–inhibitor interactions. This also indicates the specificity of the interaction of SC with IgM proteins.

With regard to the interaction of DACM-SC with dimeric IgA(Mo), we found in competitive binding experiments that the affinity decreased on introduction of DACM groups. The reason why the decrease was observed only in the interaction with dimeric IgA is unknown, but it may reflect a difference in the binding sites of the two immunoglobulin classes. The affinity of DACM-SC for dimeric IgA(Mo) was low (Table II); even when the decrease in affinity caused by DACM groups was taken into account, it was 10–20 times lower than the affinities for IgM proteins. This is consistent with reports of Brandtzaeg (1977) and Socken & Underdown (1978) of stronger noncovalent interactions of SC with IgM proteins than with dimeric IgA proteins. In experiments on the binding of rabbit SC with rabbit dimeric IgA bound to Sepharose gel, Kühn & Kraehenbuhl (1979) observed a k_1 of 2.4×10^5 M^{-1} min^{-1} and a k_{-1} of 1.8×10^{-3} min^{-1} at 23 °C. Both these values are much smaller than ours obtained for the SC–IgM interaction, but because of the difference in the immunoglobulin classes tested and the techniques used, the reason for these differences is difficult to evaluate. Kinetic studies on the interaction of DACM-SC with IgA protein are in progress and should provide corroboratory data for the rate constants obtained for IgM proteins.

Interaction of DACM-SC with the $(Fc)_{5\mu}$ Fragment. We measured the interaction of DACM-SC with $(Fc)_{5\mu}$ fragments to clarify the role of Fab'_μ regions in the SC–IgM interaction. We obtained the $(Fc)_{5\mu}$ fragments by tryptic digestion of IgM proteins at high temperature. We thought that this treatment might damage the binding site for SC and result in a substantial decrease in the affinity, but in fact we found that DACM-SC is bound to the $(Fc)_{5\mu}$ fragments with a stoichiometry of 1:1, as in intact IgM proteins. The affinity constant for the $(Fc)_{5\mu}$ fragment of IgM(Mi) was 3 times that for the intact IgM(Mi) protein. The affinity constants for the $(Fc)_{5\mu}$ fragments from the two IgM proteins were similar, being about 1.9×10^9 M^{-1}. This similarity suggests that the affinity for the $(Fc)_{5\mu}$ fragments represents the intrinsic affinity for IgM proteins and that the smaller value of K_a for IgM(Mi) than for its fragment is due to some distinct inhibition of the interaction. The rate constants are consistent with this consideration. While the values of k_1 and k_{-1} for IgM(Oda) and the $(Fc)_{5\mu}$ fragments of the two IgM proteins are very similar, those for IgM(Mi) are different, and the latter values both contribute to the decrease in the affinity.

The IgM molecule contains an additional domain, Cμ2, and it is proposed that the Fab'_μ regions, which include the Cμ2 domain, are highly flexible relative to the pentameric $(Fc)_{5\mu}$ region, which consists of Cμ3 and Cμ4 domains (Cathou, 1978). It is known that, on binding to a particulate antigen, the stellar conformation of the IgM molecule is dramatically changed to a staple conformation (Feinstein et al., 1977). Thus, the exact conformation of the IgM molecule should depend on the conditions. The $(Fc)_{5\mu}$ region is the core of the IgM molecule, but it constitutes only one-third of the molecule. Therefore, although we do not know the structure of the SC binding site in the $(Fc)_{5\mu}$ region, it is readily conceivable that the bulky Fab'_μ regions could cause steric hindrance of the interaction of SC with this region. If the conformation of the IgM molecule depends on the specimen (Cathou, 1978), the extent of this inhibition must depend on the IgM protein. In this study, inhibition was evident in the interaction with IgM(Mi), but not in that with IgM(Oda). We examined only two IgM proteins and their fragments, but to confirm the validity of our idea, it is important to study other IgM proteins. It has been proposed that the stoichiometry and the affinity of interaction of SC with polymeric immunoglobulins are closely related to the J chain content (Brandtzaeg, 1976). In this study, the role of the J chain was uncertain, because the stoichiometry of the J chain in the IgM proteins used was unknown. Thus, it is also important to study the interaction in relation to the J chain to determine whether the affinity constant obtained for the $(Fc)_{5\mu}$ fragments is the intrinsic one.

Environment of the Reactive Disulfide Bond. It is relevant to consider the environment of the reactive disulfide bond of SC because this bond plays an important role in formation of a complex with IgA. The reactivity of the disulfide bond with the reducing reagent has a close relation to the location of the bond in the protein molecule (Creighton, 1978; Goto & Hamaguchi, 1981), and the high reactivity of the reactive disulfide bond of SC indicates that it is located on the surface of the SC molecule. On reduction of this bond, the two thiol groups produced react rapidly with added DACM. The maximum wavelength (483 nm) of the fluorescence spectrum of DACM-SC is only 2 nm shorter than that of DACM attached to 2-mercaptoethanol. These results are consistent with the above idea of the surface location of the disulfide bond. When DACM-SC is bound to polymeric immunoglobulins, its fluorescence intensity and maximum wavelength change. These changes are, however, relatively small compared to the change in the spectrum of DACM attached to 2-mercaptoethanol observed when the solvent is replaced by ethanol. This indicates that the environment of the DACM groups is only slightly perturbed by the interaction. From the primary structure of the rabbit SC precursor, Mostov et al. (1984) indicated that the reactive disulfide bond is located in the fifth domain of the membrane-bound SC, corresponding to the carboxyl-terminal portion of free SC. Thus, the DACM groups in the fifth domain of the SC molecule are considered not to be buried greatly when DACM-SC is bound to polymeric immunoglobulins. The small influence of DACM on the affinity of SC for immunoglobulins supports this consideration. Cunningham-Rundles & Lamm (1975) have shown that partial reduction of secretory IgA results in specific reduction of the same reactive cysteine residues of SC, although in this case the residues are involved in interchain disulfide bonds between SC and dimeric IgA. Their results indicate exposure of the disulfide bond to the solvent, which is consistent

with our consideration. For formation of the complex of SC with dimeric IgA, which first occurs by noncovalent interaction, it should be important that the reactive disulfide bond is exposed to the solvent, thus allowing the thiol–disulfide exchange reaction to proceed rapidly, resulting in formation of interchain disulfide bonds that stabilize the complex.

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