Direct Measurement of the Weak Interactions between a Mouse Fc Receptor (FcγRII) and IgG1 in the Absence and Presence of Hapten: A Total Internal Reflection Fluorescence Microscopy Study†

Helen V. Hsieh, Claudia L. Poglitsch,‡ and Nancy L. Thompson*

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290

Received June 24, 1992; Revised Manuscript Received September 10, 1992

ABSTRACT: Total internal reflection fluorescence microscopy (TIRFM) has been used to directly measure the weak dissociation constants of IgG with a mouse IgG receptor (moFcγRII) that has been purified and reconstituted into substrate-supported planar membranes. Dissociation constants were measured for three different mouse monoclonal anti-dinitrophenyl (DNP) IgG1 antibodies and for polyclonal mouse IgG, in the absence and presence of saturating amounts of hapten (DNP-glycine). The dissociation constant for polyclonal mouse IgG was 3 μM, which agrees well with previous results. The dissociation constants for the three monoclonal antibodies with moFcγRII ranged from 2 μM to 3 μM and were not statistically different, suggesting that changes in moFcγRII dissociation constants which may exist within the IgG1 subclass are less than the error of the TIRFM measurements (~20%). The measured IgG1–moFcγRII dissociation constants were not different for individual monoclonal antibodies in the absence or presence of saturating concentrations of DNP-glycine, directly showing that possible allosteric changes which might occur upon hapten binding and affect the equilibrium characteristics of Fc receptor binding are small. This work demonstrates a new approach for quantitatively examining the effects of solution components on weak receptor–ligand interactions.

The interaction of antibody–antigen complexes with immune cells triggers critical cellular responses including endocytosis, phagocytosis, and B cell activation and regulation. These processes are mediated by the Fc regions of antibodies and by cell surface Fc receptors. There are several different forms of Fc receptors, and the different receptor forms are distributed on a variety of cell types (Unkeless et al., 1988; Mellman et al., 1988). In the mouse and human, three forms of Fc receptors for IgG have been identified (FcγRI, FcγRII, and FcγRIII) (Ravetch & Kinet, 1991). Most Fcγ receptors are glycosylated, membrane-spanning polypeptides with extracellular immunoglobulin-related domains.

Despite recent elucidation of Fcγ receptor genes and transcripts, physical characterization of IgG–Fcγ receptor interactions has been hindered by the multiple receptor forms found on different cell types and by the weak association between monomeric IgG and FcγRII or FcγRIII. The previous use of multivalent IgG complexes in direct binding measurements (or in competition with monomeric IgG), and the concurrent presence (in some cases) of high-affinity Fcγ receptors, has led to ambiguities in determining IgG–Fcγ receptor binding characteristics (Segal & Titus, 1978; Dower et al., 1981a,b). Direct examinations of the weak association of monomeric IgG with FcγRII and FcγRIII are ultimately important in delineating the specificity and dynamics of the complete set of events occurring at the membrane surface that leads to cellular response.

This paper describes the direct measurement of the weak dissociation constants for three different mouse monoclonal anti-dinitrophenyl (DNP) IgG1 antibodies, with a mouse Fcγ receptor (moFcγRII), in the absence and presence of saturating amounts of hapten (DNP-glycine). These measurements allow comparison of the dissociation constants of different monomeric monoclonal antibodies within the same IgG subclass with moFcγRII, and also provide a direct means to evaluate whether bound, monovalent hapten alone can influence the interaction between IgG1 and moFcγRII. The measurements were carried out by reconstituting purified moFcγRII into substrate-supported planar membranes (McConnell et al., 1986) and using the technique of total internal reflection fluorescence microscopy (TIRFM) (Poglitsch et al., 1991; Kalb et al., 1990).

MATERIALS AND METHODS

Cells. J774A.1, a macrophage-like cell line containing cell surface moFcγRII, was obtained from the University of North Carolina Tissue Culture Facility. 2.4G2, a rat–mouse hybridoma which secretes antibodies specific for moFcγRII (Unkeless, 1979) was provided by B. Diamond of the Albert Einstein College of Medicine. Three different mouse–mouse hybridomas which produce anti-DNP IgG1 were obtained from the following sources: ANO2, H. M. McConnell, Stanford University (Leahy et al., 1988), DHK109.3, N. R. Klinman, Scripps Clinic and Research Foundation (Liu et al., 1980), and 1B7.11, American Type Culture Collection (Hay et al., 1992). Hybridomas were maintained in culture flasks; the J774A.1 cell line was maintained in spinner flasks. Cells were grown in media supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 100 IU penicillin, 100 μg/mL streptomycin, and fetal calf serum (FCS) that had been heat inactivated for 30 min at 56 °C, as follows: J774A.1, ANO2, and 1B7.11, DMEM/F12, 5% FCS; 2.4G2, DMEM/F12, 10% FCS; DHK109.3, RPMI 1640, 1% FCS.

Antibodies. 2.4G2 antibodies were purified from cell supernatants by affinity chromatography with anti-(rat IgG κ light chain) antibodies, and 2.4G2 Fab were produced and

† This work was supported by National Institutes of Health Grant GM37145.

* To whom correspondence should be addressed.

‡ Present address: Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305-5400.

0006-2960/92/0431-11562$03.00/0

isolated as described (Poglitsch & Thompson, 1990). Intact polyclonal mouse IgG was obtained commercially (Jackson ImmunoResearch, Inc., West Grove, PA). ANO2, 1B7.11, and DHK109.3 antibodies were purified from cell supernatants by affinity chromatography with DNP-conjugated human serum albumin (Pisarchick & Thompson, 1990). The immunoglobulin subclass of antibodies isolated from ANO2, 1B7.11, and DHK109.3 supernatants was verified to be IgG1 (M. T. Sumner & N. L. Thompson, unpublished results) with the EIA Grade Mouse Typer System (Bio-Rad Laboratories, Richmond, CA). This analysis also indicated that the polyclonal mouse IgG consisted primarily of IgG1.

Antibodies were labeled with tetramethylrhodamine 5(and-6)-isothiocyanate (Molecular Probes, Inc., Junction City, OR) as previously described (Timbs & Thompson, 1990) and dialyzed against phosphate-buffered saline (PBS; 0.05 M sodium phosphate, 0.15 M sodium chloride, 0.01% sodium azide, pH 7.4). The labeling ratios and antibody concentrations were determined spectrophotometrically. The molar ratios of fluorophore to antibody were 0.7–2.0 for the monoclonal IgG1 antibodies and less than one for the polyclonal mouse IgG. The spectrophotometrically-measured antibody concentrations agreed within 10% with independent determinations from a bicinchoninic acid (BCA) assay (Pierce Chemical Co., Rockford, IL) in which unlabeled antibodies were standards for labeled (R-) antibodies and bovine serum albumin (BSA, Sigma Chem. Co., St. Louis, MO) was the standard for unlabeled antibodies.

Labeled antibodies were clarified (100000*g*, 2 h, 4 °C) no more than 24 h prior to application to planar membranes. Gel filtration measurements (G200-120 Sephadex; 1.5 cm × 69 cm; flow rate, 0.1 mL/min; sample volume, 1.0 mL; PBS; 25 °C) showed that all labeled antibodies (1.0 mg/mL) eluted with symmetrical peaks and at identical elution volumes, corresponding to IgG monomers. Possible higher molecular weight IgG aggregates were not detected.

Planar Membranes. MoFcγRII was purified from homogenized J774A.1 cells by 2.4G2 Fab affinity chromatography (Poglitsch et al., 1991; Mellman & Unkeless, 1980). The purity of the product was estimated with sodium dodecyl sulfate–polyacrylamide gel electrophoresis and silver staining. The BCA assay was used to determine the yield (usually ≈70 μg from 2 × 10^9 cells). Previous N-terminal sequence determinations have confirmed that the purified product was moFcγRII.

Purified moFcγRII was reconstituted into vesicles by detergent dialysis as described (Poglitsch et al., 1991), except that the lipid films were dried under vacuum for 4 h. The phospholipid composition was egg phosphatidylcholine:cholesterol (Sigma Chem. Co., St. Louis, MO) 6:1 (w:w), and the protein:lipid ratio was 1:10 (w:w). Vesicles were also prepared without moFcγRII.

Membranes were formed on planar fused silica surfaces by vesicle adsorption and fusion as described (Poglitsch et al., 1991), except that the substrates were washed with 3 mL of PBS after vesicle adsorption. Planar membranes were treated with 10 mg/mL BSA/PBS (65 μL, 30 min) to block nonspecific binding sites and then with labeled antibodies in 10 mg/mL BSA/PBS (250 μL, 30 min). Some antibody solutions also contained 0.25 μM 2.4G2 Fab and/or 100 μM DNP-glycine.

Fluorescence Spectroscopy and Microscopy. Solution dissociation constants for IgG1 antibodies and DNP-glycine in solution were measured with ultraviolet fluorescence quenching (SLM 8000C, λ_{ex} = 280 nm, λ_{em} = 340 nm) (Pisarchick & Thompson, 1990) at room temperature. Each quenching curve was corrected for collisional quenching using data for tetramethylrhodamine-labeled polyclonal mouse IgG. The relative tetramethylrhodamine fluorescence for antibodies at different dye/antibody labeling ratios was measured with fluorescence spectroscopy (SLM 8000C, λ_{ex} = 514 nm, λ_{em} = 540–625 nm).

The fluorescence originating from labeled antibodies on planar membranes was measured at room temperature with TIRFM (Pisarchick & Thompson, 1990). The fluorescence microscope was composed of an argon ion laser (Innova 90-3, Coherent, Inc., Palo Alto, CA), an inverted optical microscope (Zeiss IM-35, Eastern Microscope Co., Raleigh, NC), and a single photon-counting photomultiplier (RCA 31034A, Lancaster, PA) interfaced to an IBM PC AT. For each sample type, nine spatially independent measurements were obtained on each of two or three planar membranes formed from at least two separate vesicle preparations.

RESULTS

Specificity of IgG1 Binding to moFcγRII(+) Membranes. TIRFM was used to measure the evanescently excited fluorescence on planar membranes with (+) and without (–) moFcγRII. The fluorescence was measured as a function of the solution concentration of tetramethylrhodamine-labeled antibodies. Representative binding curves for R-DHK109.3 are shown in Figure 1a. The fluorescence on moFcγRII(+) membranes was higher and began to saturate with increasing antibody solution concentration, whereas the fluorescence on moFcγRII(–) membranes was lower and linear with the antibody solution concentration. This result suggests that a significant fraction of the fluorescence on moFcγRII(+) membranes arose from labeled antibodies bound to the reconstituted moFcγRII.

As a control, the moFcγRII(+) membranes were treated with solutions containing both labeled antibodies and an excess (0.25 μM) of 2.4G2 Fab. 2.4G2 is an anti-moFcγRII antibody that competes with IgG for binding to moFcγRII (dissociation constant ≈ 1 nM) (Mellman & Unkeless, 1980). Previous work has shown that 2.4G2 Fab binds tightly and specifically to moFcγRII in supported planar membranes (Poglitsch et al., 1991). The fluorescence for the samples containing 2.4G2 Fab was approximately equal to the fluorescence on moFcγRII(–) membranes for all antibodies. This result indicates that the amount of fluorescence on moFcγRII(+) membranes due to fluorescent antibodies in solution but within the evanescent field and/or to nonspecifically bound antibodies was equal to the fluorescence measured on moFcγRII(–) membranes. Thus, the fluorescence on moFcγRII(+) membranes due only to antibodies specifically bound to moFcγRII was calculated as the difference of the fluorescence on moFcγRII(+) and moFcγRII(–) membranes (Figure 1b).

IgG1–moFcγRII Dissociation Constants Measured by TIRFM. To measure the apparent IgG–moFcγRII dissociation constants, the fluorescence difference data were curve-fit to the theoretical form for a bimolecular reaction occurring at the surface:

$$F([A]) = \frac{F(\infty)[A]}{K_{d1} + [A]} \quad (1)$$

In this analysis, the antibody solution concentration, [A], and the surface fluorescence, $F([A])$, were experimentally determined parameters; and the apparent dissociation constant, K_{d1}, and the fluorescence at infinite antibody solution concentration, $F(\infty)$, were free parameters.

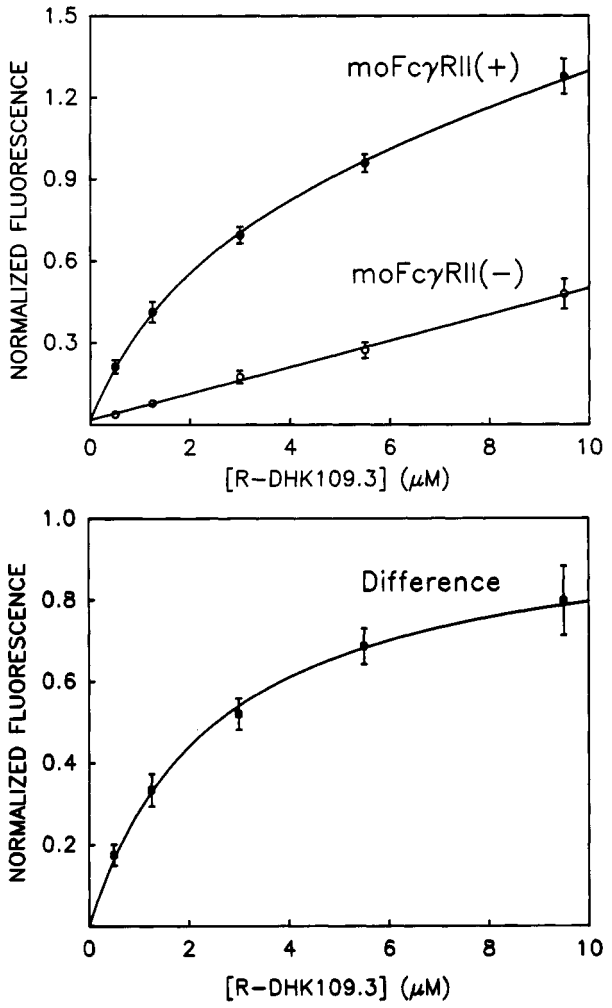

FIGURE 1: Representative binding curve for tetramethylrhodamine-labeled antibodies on planar membranes. (a, top) The evanescently excited fluorescence on moFcγRII(+) membranes (●) and on moFcγRII(−) membranes (○) is plotted as a function of the solution concentration of R-DHK109.3. The moFcγRII(−) data are compared to the best fit to a straight line. The moFcγRII(+) data are compared to the sum of this straight line and the curve shown in (b). (b, bottom) The difference (■) of the fluorescence on moFcγRII(+) and moFcγRII(−) membranes is plotted versus antibody concentration. The best fit to eq 1 gave K_{d1} = 2.6 μM. The fluorescence has been normalized so that the theoretical value of $F(\infty)$ equals one. Uncertainties are standard errors in the means for averages over nine spatially independent measurements on each of three planar membranes.

Table I: Membrane Dissociation Constants for IgG and MoFcγRII[a]

antibody	K_{d1} (μM): DNP-G (−)	K_{d1} (μM): DNP-G (+)	K_{d1} (μM): DNP-G (±)
1B7.11	3.4 ± 1.4 (3)	2.9 ± 0.9 (3)	3.1 ± 0.8 (6)
ANO2	1.4 ± 0.2 (4)	3.0 ● 1.1 (4)	2.2 ± 0.7 (8)
DHK109.3	2.6 ± 0.2 (3)	2.2 ± 0.3 (3)	2.4 ± 0.3 (6)
Mouse IgG	3.2 ± 0.4 (4)	3.5 ± 0.1 (2)	3.3 ± 0.3 (6)

[a] The IgG1–moFcγRII dissociation constants, K_{d1}, were measured in the absence and presence of 100 μM DNP-glycine. The means and standard deviations of the means for measurements on planar membranes made from different vesicle preparations are shown. The values in parentheses are the numbers of independently measured binding curves.

This analysis gave dissociation constants for the three different monoclonal IgG1 antibodies and for polyclonal mouse IgG (Table I). The best-fit values of K_{d1} for the IgG1 antibodies ranged from 1.4 μM to 3.4 μM, and the K_{d1} for polyclonal mouse IgG was 3.2 μM.

Table II: Solution Dissociation Constants for IgG1 and DNP-Glycine[a]

antibody	K_{d2} (μM)	Q_b/Q_f	f	ref
1B7.11	0.15 ± 0.01	0.30 ± 0.03	>0.99	*b*
R-1B7.11	0.17 ± 0.04	0.30 ± 0.04	>0.99	
ANO2	0.36 ± 0.03	0.41 ± 0.01	>0.99	*c*
R-ANO2	0.33 ± 0.03	0.47 ± 0.01	>0.99	*c*
DHK109.3	4.6 ● 0.3	0.27 ± 0.01	0.94	*b*
R-DHK109.3	5.1 ± 0.1	0.26 ± 0.01	0.95	

[a] The solution dissociation constants, K_{d2}, were measured with ultraviolet fluorescence quenching. The values of Q_b/Q_f are the ratios of ultraviolet fluorescence intensities of bound and free antibodies. The fractions of the antigen binding sites that were occupied at 100 μM DNP-glycine, f, were calculated from eq 2. Values for K_{d2} and Q_b/Q_f are the means and standard deviation of the means obtained from measurements of two or three independent quenching curves. Some values are quoted from other studies. [b] M. T. Sumner & N. L. Thompson, unpublished data. [c] Pisarchick & Thompson, 1990.

T-tests were used to compare the different dissociation constants. As there was no statistical difference between the K_{d1} values in the absence and presence of DNP-glycine (see below), comparisons were made using the K_{d1} values calculated from all binding curves (with and without DNP-glycine). The *p* levels for which the K_{d1} values for the three IgG1 antibodies were significantly different ranged from 0.35 to 0.78, demonstrating that within experimental error the measured values of K_{d1} were not different for the three monoclonal anti-DNP IgG1 antibodies. In comparing the K_{d1} values for the IgG1 antibodies with the K_{d1} value of polyclonal mouse IgG, the *p* levels ranged from 0.03 to 0.80, with one IgG1 antibody (DHK109.3) possibly differing from polyclonal mouse IgG.

Surface-Site Densities for Different IgG1 Antibodies. The surface-associated fluorescence at a high (5 μM) antibody solution concentration was measured for the different tetramethylrhodamine-labeled monoclonal antibodies on moFcγRII(+) planar membranes made from the same preparation of vesicles. The measurements were corrected for the spectrofluorometrically determined relative fluorescence intensities of different labeled antibody preparations (which resulted primarily from different molar dye/antibody ratios). The corrected, surface fluorescence values at the high antibody solution concentration (determined for two independent vesicle preparations) agreed within 20% for the different monoclonal antibodies. Similar results were also measured for moFcγRII(−) membranes. These measurements indicate that the three monoclonal IgG1 antibodies recognize the same number of discrete surface binding sites. Previous estimates have implied that the planar membranes contain ~5000 receptors/μm², that approximately one-third of the receptors bind 2.4G2 Fab, that 25–50% of the receptors that bind 2.4G2 Fab also bind IgG, and that all of the receptors that bind IgG also bind 2.4G2 Fab (Poglitsch et al., 1991). Thus, the density of active moFcγRII in the planar membranes was ~600 receptors/μm².

Effect of Saturating Amounts of DNP-Glycine on the IgG1–moFcγRII Dissociation Constants. The dissociation constant K_{d2} for each monoclonal IgG1 antibody with DNP-glycine in solution was measured with ultraviolet fluorescence quenching (Table II) as previously described (Pisarchick & Thompson, 1990). The measured values of K_{d2} ranged from 0.2 μM (1B7.11) to 5 μM (DHK109.3). The K_{d2} values were not affected by the presence of the tetramethylrhodamine label.

The measured values of K_{d2} were used to determine a DNP-glycine concentration at which most of the DNP-glycine binding sites would be occupied. The fraction of antibody

binding sites occupied with DNP-glycine is given by

$$f = \frac{[T] + 2[A] + K_{d2} - \sqrt{([T] + 2[A] + K_{d2})^2 - 8[A][T]}}{4[A]} \quad (2)$$

where [T] and [A] are the total solution concentrations of DNP-glycine and antibodies, respectively. Equation 2 implies that 100 μM DNP-glycine saturates ≥94% of the antibody binding sites (Table II). Spectrofluorometric measurements indicated that 100 μM DNP-glycine had a negligible effect on the intensity of the tetramethylrhodamine fluorescence (data not shown).

TIRFM was used to measure the dissociation constants, K_{d1}, for antibodies with moFcγRII in planar membranes, in the absence and presence of saturating amounts of DNP-glycine (Table I). The values of K_{d1} for polyclonal mouse IgG were compared using unpaired *t*-tests. These calculations revealed no difference in K_{d1} for the control polyclonal mouse IgG which has no specificity for DNP-glycine ($p = 0.69$). The dissociation constants for each monoclonal antibody in the absence and presence of DNP-glycine were compared using paired *t*-tests, for the different sets of jointly-measured binding curves (one with and one without DNP-glycine). These calculations indicated no differences in K_{d1} in the absence and presence of DNP-glycine; the *p* levels ranged from 0.14 to 0.46.

DISCUSSION

The dissociation constants for IgG1 and moFcγRII measured with TIRFM ranged from 2.2 μM to 3.1 μM. These values agree well with earlier indirectly measured values for two mouse myeloma IgG1 antibodies on macrophage cell surfaces (3.6 μM) (Segal & Titus, 1978). The dissociation constant for polyclonal mouse IgG (which was found to consist primarily of IgG1) was equal to 3.3 μM. Earlier results for polyclonal mouse IgG using TIRFM in both direct and competition measurements have also yielded similar values for the IgG–moFcγRII dissociation constants (Poglitsch et al., 1991; Poglitsch & Thompson, 1990).

The observations that the IgG subclass specificities of different Fcγ receptors vary, that the expression of Fcγ receptors is cell-type specific, and that different effector functions are associated with different cell types (Ravetch & Kinet, 1991; Unkeless et al., 1988; Mellman et al., 1988; Weinshank et al., 1988) suggest that the subclass specificity of Fcγ receptors is of physiological significance. For example, previous work has directly shown that some IgG-mediated immune responses can be subclass restricted for some categories of antigen (Perlmutter et al., 1978; Coutelier et al., 1991).

In most earlier studies where the IgG subclass specificity of Fcγ receptors has been defined, either polyclonal IgG or only one monoclonal antibody has been used (e.g., Schneider et al., 1981; Dower et al., 1981a,b). Because, in general, the affinity for Fcγ receptors within an IgG subclass might vary (perhaps arising from differences in glycosylation or segmental flexibility), we have compared the binding of three different monoclonal antibodies within a given subclass (IgG1). As shown in Table I, the moFcγRII dissociation constants for the three different IgG1 monoclonal antibodies were equivalent. Because all of the antibodies have the BALB/c allotype (Igh-4^a; Balakrishnan et al., 1982; Liu et al., 1980; Hay et al., 1992; Goding, 1986), the result that the values of K_{d1} were equivalent implies that any small differences which may exist in IgG1–moFcγRII dissociation constants within a given allotype are less than the error of the TIRFM measurements (~20%).

The question of variability in IgG binding to Fc receptors within a given IgG subclass has been indirectly (and qualitatively) addressed in other contexts. In one study (Kimura et al., 1986), the antibody-dependent dissociation of mouse macrophage cells from supported planar membranes containing hapten-conjugated phospholipids was examined with a panel of eight anti-hapten monoclonal antibodies. Macrophage binding via IgG1 antibodies to the planar membranes varied little for seven of the antibodies but was much lower for one of the antibodies (1B7). The 1B7 antibody is not equivalent to the 1B7.11 antibody used in this work (M. Nakanishi, personal communication). In another system, polyclonal rat IgG2a and IgG2b were found to bind to isolated rat Fcγ receptor more tightly than their monoclonal counterparts (Hobbs et al., 1987).

Although a significant body of work provides evidence for the lack of major conformational changes in IgG upon binding to hapten or antigen (Metzger, 1978), several studies have suggested that IgG may be structurally altered in more minor ways by the presence of bound antigen (Colman, 1988). For example, indirect evidence for conformational changes in the Fc region of IgG upon the binding of haptens (Okada et al., 1985) or larger monovalent antigens (Schlessinger et al., 1975) has been presented; and new antigenic determinants not present in monomeric or heat-aggregated IgG can be induced in the Fcγ region when it is in the form of immunocomplexes (Brown & Bekisz, 1984). The effect of occupation of the antigen binding site on Fc-mediated effector functions has not previously been resolved (Burton, 1990), in part because previous cell binding measurements with polymeric immune complexes could not isolate the effect of IgG binding site occupation versus that of multivalency.

The dissociation constants measured for anti-dinitrophenyl antibodies with moFcγRII(+) membranes were not different in the presence and absence of saturating amounts of DNP-glycine (Table I). This finding is in agreement with results obtained with X-ray crystallography (Novotny et al., 1989) and circular polarization of fluorescence (Schlessinger et al., 1975), which indicate that the binding of small, monovalent haptens is not accompanied by a significant conformational change in IgG. The finding also agrees with previous work showing that bound, monovalent haptens do not alter other IgG effector functions (Pecht et al., 1977). That antigenic ligands larger than haptens may exert a more prominent effect is plausible on the basis of crystallographic and NMR studies which have demonstrated the antibody–ligand contact area to be larger and different in character for protein and some peptide ligands in contrast to small, organic haptens (Brünger et al., 1991; Zilber et al., 1990).

A related question is whether the association of IgG and moFcγRII affects the affinity of IgG for DNP-glycine. Because the interactions between DNP-glycine, IgG, and moFcγRII may be viewed as a cyclic reaction, this question is indirectly addressed from the direct measurements of the IgG1–moFcγRII dissociation constants (Creighton, 1984). Indeed, the experimental result that DNP-glycine does not change the IgG1–moFcγRII dissociation constant implies that the affinity of IgG for DNP-glycine is equivalent for IgG in solution and for receptor-bound IgG. This result agrees with previous measurements for an anti-dinitrophenyl IgE antibody in solution and bound to the high-affinity Fcε receptor on rat basophil leukemia cells (Erickson et al., 1986).

Two recently developed techniques, the formation of substrate-supported planar membranes and total internal reflection fluorescence microscopy, have been combined and used to measure the relatively weak dissociation constants of IgG with moFcγRII. This work demonstrates that this approach can be used to systematically examine the effects of solution components on weak receptor–ligand interactions. In the future, using TIRFM with techniques such as fluorescence photobleaching recovery or correlation spectroscopy should also provide dynamic information such as kinetic rate constants.

ACKNOWLEDGMENT

We thank Harden M. McConnell of Stanford University for providing ANO2 hybridoma cells, Norman R. Klinman of the Scripps Research Institute for DHK109.3 hybridoma cells, Betty Diamond of the Albert Einstein College of Medicine for 2.4G2 hybridoma cells, and Lori Coe, Sheila Jones, and Martina T. Summer for assistance with biochemical preparations.

REFERENCES

Balakrishnan, K., Hsu, F. J., Hafeman, D. G., & McConnell, H. M. (1982) *Biochim. Biophys. Acta 721*, 30–38.

Brown, E. J., & Bekisz, J. (1984) *J. Immunol. 132*, 1346–1352.

Brünger, A. T., Leahy, D. J., Hynes, T. R., & Fox, R. O. (1991) *J. Mol. Biol. 221*, 239–256.

Burton, D. R. (1990) *Trends Biochem. Sci. 15*, 64–69.

Colman, P. M. (1988) *Adv. Immunol. 43*, 99–132.

Coutelier, J.-P., Van der Logt, J. T. M., & Heessen, F. W. A. (1991) *J. Immunol. 147*, 1383–1386.

Creighton, T. E. (1984) *Proteins: Structure and Molecular Principles*, W. H. Freeman and Company, New York, NY, pp 346–347.

Dower, S. K., DeLisi, C., Titus, J. A., & Segal, D. M. (1981a) *Biochemistry 20*, 6326–6334.

Dower, S. K., Titus, J. A., DeLisi, C., & Segal, D. M. (1981b) *Biochemistry 20*, 6335–6340.

Erickson, J., Kane, P., Goldstein, B., Holowka, D., and Baird, B. (1986) *Mol. Immunol. 23*, 769–781.

Goding, J. W. (1986) *Monoclonal Antibodies: Principles and Practice*, Harcourt Brace Jovanovich, New York, NY.

Hay, R., Caputo, J., Chen, T. R., Macy, M., McClintock, P., & Reid, Y., Eds. (1992) *American Type Culture Collection Catalogue of Cell Lines and Hybridomas*, 7th ed., American Type Culture Collection, Rockville, MD.

Hobbs, S. M., Jackson, L. E., & Peppard, J. V. (1987) *J. Biol. Chem. 262*, 8041–8046.

Kalb, E., Engel, J., & Tamm, L. K. (1990) *Biochemistry 29*, 1607–1613.

Kimura, K., Nakanishi, M., Ueda, M., Ueno, J., Nariuchi, H., Furukawa, S., & Yasuda, T. (1986) *Immunology 59*, 235–238.

Leahy, D. J., Rule, G. S., Whittaker, M. M., & McConnell, H. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 3661–3665.

Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C., Sherman, L. A., Klinman, N. R., & Katz, D. H. (1980) *J. Immunol. 124*, 2728–2737.

McConnell, H. M., Watts, T. H., Weis, R. M., & Brian, A. A. (1986) *Biochim. Biophys. Acta 864*, 95–106.

Mellman, I. S., & Unkeless, J. C. (1980) *J. Exp. Med. 152*, 1048–1069.

Mellman, I., Koch, T., Healey, G., Hunziker, W., Lewis, V., Plutner, H., Miettinen, H., Vaux, D., Moore, K., & Stuart, S. (1988) *J. Cell Sci. Suppl. 9*, 45–65.

Metzger, H. (1978) *Contemp. Top. Mol. Immunol. 7*, 119–152.

Novotny, J., Bruccoleri, R. E., & Saul, F. A. (1989) *Biochemistry 28*, 4735–4749.

Okada, A., Nakanishi, M., Tsurui, H., Wada, A., Terashima, M., & Osawa, T. (1985) *Mol. Immunol. 22*, 715–718.

Pecht, I., Ehrenberg, B., Calef, E., & Arnon, R. (1977) *Biochem. Biophys. Res. Commun. 74*, 1302–1310.

Perlmutter, R. M., Hansburg, D., Briles, D. E., Nicolotti, R. A., & Davie, J. M. (1978) *J. Immunol. 121*, 566–572.

Pisarchick, M. L., & Thompson, N. L. (1990) *Biophys. J. 58*, 1235–1249.

Poglitsch, C. L., & Thompson, N. L. (1990) *Biochemistry 29*, 248–254.

Poglitsch, C. L., Sumner, M. T., & Thompson, N. L. (1991) *Biochemistry 30*, 6662–6671.

Ravetch, J. V., & Kinet, J.-P. (1991) *Annu. Rev. Immunol. 9*, 457–492.

Schlessinger, J., Steinberg, I. Z., Givol, D., Hochman, J., & Pecht, I. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2775–2779.

Schneider, R. J., Atkinson, J. P., Krause, V., & Kulczycki, A., Jr. (1981) *J. Immunol. 126*, 735–740.

Segal, D. M., & Titus, J. A. (1978) *J. Immunol. 120*, 1395–1403.

Timbs, M. M., & Thompson, N. L. (1990) *Biophys. J. 58*, 413–428.

Unkeless, J. C. (1979) *J. Exp. Med. 150*, 580–596.

Unkeless, J. C., Scigliano, E., & Freedman, V. H. (1988) *Annu. Rev. Immunol. 6*, 251–281.

Weinshank, R. L., Luster, A. D., & Ravetch, J. V. (1988) *J. Exp. Med. 167*, 1909–1925.

Zilber, B., Scherf, T., Levitt, M., & Anglister, J. (1990) *Biochemistry 29*, 10032–10041.