Equilibrium and Kinetic Aspects of the Interaction of Isolated Variable and Constant Domains of Light Chain with the Fd′ Fragment of Immunoglobulin G†

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ABSTRACT: The noncovalent interaction of κ light chains and fragments corresponding to their variable (V_κ) and constant (C_κ) domains with Fd′ fragments of immunoglobulin G has been studied by ultraviolet difference spectroscopy in 4 mM sodium acetate buffer (pH 5.4). At the concentrations used, the intact κ chains, the V_κ fragments, and Fd′ existed as monomer–dimer equilibrium mixtures, and the C_κ fragments were monomeric. Characteristic red-shifted difference spectra were obtained when V_κ and C_κ were recombined separately with Fd′, suggesting that aromatic chromophores were transferred to a nonpolar environment. When these spectra were summed, significant differences were noted when compared to the spectrum obtained when the parent κ chain was recombined with Fd′. In contrast, when V_κ and C_κ were simultaneously bound to Fd′, the spectrum was identical with that observed with the intact κ chain. When either V_κ or C_κ fragments were recombined with a binary complex formed with Fd′ and the complementary domain fragment, significantly different spectra were observed. Equilibrium binding curves were constructed from the spectral data indicating a 1:1 binding between Fd′ and both V_κ and C_κ. The fragments bound to Fd′ with substantially lower affinity than that for the parent κ chain. The affinity of V_κ was enhanced in the presence of C_κ. The time dependence of the spectral changes was used to determine the rates of domain association. All reactions were second order. The forward rate constant for the binding of V_κ to Fd′ was the same as for the intact κ chain and was not affected by the presence of C_κ ($\sim$200 M^{-1} s^{-1}). C_κ was bound much more slowly ($\sim$10 M^{-1} s^{-1}) although the rate was significantly increased in the presence of V_κ ($\sim$70 M^{-1} s^{-1}). Although the C_κ fragments from several different κ chains bound to Fd′ in a similar way, only V_κ from the autologous κ chain showed significant affinity for Fd′. However, in the presence of C_κ this specific interaction was no longer apparent and heterologous V_κ fragments were capable of binding to Fd′. The data presented suggest (1) that the high-affinity association of Fd′ and κ chain derives from a combination of relatively weak interactions involving pairs of domains (i.e., V_H–V_κ and $C_\gamma1$–C_κ) and (2) that the binding of one κ-chain domain to Fd′ induces a conformational change in the adjacent domain in Fd′, thus modifying its reactivity toward the complementary κ-chain domain. It also appears that the specific association of autologous V_κ and V_H may be due to quaternary interactions between hypervariable sequence regions or substitutions in the framework of the variable regions.

The immunoglobulin G (IgG)[1] molecule is composed of two identical γ chains (M_r 53 000) and two identical L chains (M_r 22 500). Each chain is folded into a number of compact, globular domains, each corresponding to a stretch of about 110 amino acid residues: V_L and C_L in the L chain and V_H, $C_\gamma1$, $C_\gamma2$, and $C_\gamma3$ in the γ chain [for a review, see Cathou & Dorrington (1975)]. High-resolution X-ray crystallography has provided detailed information concerning the noncovalent quaternary association between domains of different chains (trans interactions) as well as between domains within each type of chain (cis interactions) [for reviews, see Davies et al. (1975a,b) and Huber et al. (1976)]. The four compact domains constituting the Fab fragment of IgG (i.e., V_H, $C_\gamma1$, V_L, and C_L) have similar basic secondary and tertiary structures. The molecular envelope of each domain is approximately cylindrical with four antiparallel β-pleated sheets forming one side and three sheets forming the other. Noncovalent association between the C_L and $C_\gamma1$ domains involves the interaction between the hydrophobic four-chain layers of each domain. In contrast, association between V_L and V_H involves the three-chain layers of each domain which brings the complementarity-determining ("hypervariable") regions together to form the antibody combining site. Cis interactions between domains within the same chain are much less intimate and involve only a limited number of residues.

The noncovalent recombination between autologous[2] and heterologous γ and L chains has been extensively studied in vitro. Such studies have shown that the γ–L interaction is strongly exothermic (Dorrington & Kortan, 1974; Bigelow et al., 1974), follows second-order kinetics (Bigelow et al., 1974; Azuma et al., 1975; Bunting et al., 1977; Friedman et al., 1977), and is accompanied by conformational changes in both subunits (Stevenson & Dorrington, 1970; Dorrington & Smith, 1972). The interaction has a high affinity with the association constant governing the reaction $\gamma + L \rightleftharpoons \gamma L$ estimated to be greater than 10^{10} M^{-1} (Bigelow et al., 1974). The recombined molecules resemble the native IgG in terms of their isotypic (Sher et al., 1971; Huser et al., 1971) and allotypic (Polmar & Steinberg, 1969) antigenic determinants and their physico-chemical properties (Stevenson & Dorrington, 1970; Bjork & Tanford, 1971; Dorrington & Smith, 1972).

In vitro reassociation experiments have also uncovered the phenomenon of "preferential recombination" (Grey & Mannik, 1965; Mannik, 1967; Gergerly et al., 1973; Stevenson & Mole, 1974; de Preval & Fougereau, 1976). Briefly, when

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[1] Abbreviations used: IgG, immunoglobulin G; γ, heavy chain of IgG; L, light chain; κ and λ, isotypes of L chain; V, variable region; C, constant region.

[2] "Autologous" refers to reactions between isolated γ and L chains derived from the same myeloma (monoclonal) protein, and "heterologous" describes reactions between chains derived from different myeloma proteins.

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γ chains were allowed to interact with a mixture of the autologous L chain and a heterologous L chain, each labeled with a different radioisotope of iodine, the proportion of recombinant molecules composed of autologous chains was frequently greater than expected if random association had occurred. This specific recognition between chains did not correlate with the L-chain isotype, the allotype, or the variable-region subgroup. It could not be explained in terms of differences in the rate at which the autologous and the heterologous L chains interacted with γ chains (Bunting et al., 1977). It has been suggested that preferential recombination between autologous chains may reflect subtle differences in the contact surfaces between V_H and V_L.

The present study was initiated to explore the relative importance of V_L–V_H and C_L–$C_\gamma 1$ interactions in the overall association between γ and L. Several investigators had shown previously that fragments corresponding to the V_L and C_L domains could be isolated following limited proteolytic cleavage of L chains (Solomon & McLaughlin, 1969; Karlsson et al., 1972; Seon et al., 1972). In addition, noncovalent association between isolated L-chain domains and γ chains (Smith & Dorrington, 1972; Karlsson, 1972) and between isolated V_L and V_H (Hochman et al., 1973) had been shown to be feasible. Since the COOH-terminal halves of the γ chains are not involved in γ–L interaction, we decided to study the interaction of peptic Fd′ fragment (corresponding to the NH_2-terminal half of γ chain) with isolated V_L and C_L. Previous studies had shown that the intact L chain interacted with Fd′ in an identical fashion as it did with the γ chain (Bigelow et al., 1974). As the study progressed it became apparent that this system could also provide information on the preferential interaction between V_L and V_H and on the influence one domain had on the binding of the other. The results of these experiments are also presented.

Materials and Methods

Preparation and Purification of Proteins, Subunits, and Fragments. IgG_κ monoclonal proteins were isolated from the sera of several patients with multiple myeloma by ammonium sulfate precipitation, followed by ion-exchange chromatography on a column of DEAE-cellulose (Whatman DE52) equilibrated in 20 mM NaCl and 10 mM Tris-HCl buffer, pH 7.8. The IgG was reduced with 10 mM dithioerythritol (DTE; Sigma) at pH 8.6 for 30 min at room temperature under nitrogen and alkylated with 24 mM iodiacetamide (Sigma; twice recrystallized). γ and L chains were separated on a column of Sephadex G-100 in 1.0 M propionic acid and 25 mM NaCl.

$F(ab')_2$ fragments were prepared by digesting IgG with pepsin (Worthington, Freehold, NJ) according to the method of Turner et al. (1970). The fragments were purified by filtration through a *Staphylococcus aureus* Protein A–Sepharose CL-4B column (Pharmacia Fine Chemicals, Dorval) and subsequent gel filtration on a column of Sephadex G-150 equilibrated in 0.15 M NaCl and 10 mM Tris-HCl buffer, pH 7.8 (TBS). The $F(ab')_2$ fragments were reduced and alkylated as described above, and the Fd′ fragment was separated from the L chain on a column of Sephadex G-100 equilibrated in 1.0 M propionic acid and 25 mM NaCl (Bigelow et al., 1974).

The separated γ and L chains and the Fd′ fragments were progressively renatured by extensive dialysis first against distilled water and then against 4 mM sodium acetate buffer, pH 5.4 (Stevenson & Dorrington, 1970).

Fragments corresponding to the variable and constant domains of the κ chains were obtained by limited proteolytic cleavage with either pepsin (Worthington) or trypsin (Worthington; TPCK treated) at 37 °C. Conditions giving optimal yields of the fragments for each κ chain were determined by varying times of digestion and enzyme-to-substrate ratios for both enzymes. With trypsin, the κ chain at 5 mg/mL of 10 mM Tris-HCl buffer, pH 8.0, was exposed to enzyme for between 20 and 60 min at enzyme-to-substrate ratios between 1:50 and 1:100 (w/w). Digestion was terminated by addition of a molar excess of soybean trypsin inhibitor (Sigma; 1.5:1 w/w). Peptic cleavage was performed on the κ chain at 5 mg/mL of 25 mM sodium acetate buffer, pH 4.5, for 30–60 min at enzyme-to-substrate ratios between 1:50 and 1:100 (w/w). Proteolysis was stopped by raising the pH to 8.6 by adding 4.0 M Tris. Any precipitate which formed during proteolysis was removed by centrifugation. The progress of digestion with both enzymes was followed by cellulose–acetate electrophoresis at pH 8.6.

Variable-region fragments were isolated by DEAE-cellulose chromatography (Whatman DE52) in 5 mM Tris-HCl, pH 8.2, followed by gel filtration on Sephadex G-75 in TBS. The monomeric constant-region fragments were purified by Sephadex G-75 chromatography in TBS. The purified fragments were dialyzed in Spectrapor tubing (Spectrum Medical Industries, Los Angeles, CA) against 4 mM sodium acetate buffer, pH 5.4, just prior to recombination experiments.

Difference Spectroscopy. Absorption difference spectra generated between 320 and 250 nm, when Fd′ fragment interacted with intact L chain or either one of the two domain fragments, were recorded with a Cary 118 double-beam spectrophotometer (Varian Instruments). Full-scale (10 in.) settings of either 0.02 or 0.05 absorbancy unit were used. The temperature in the cuvettes was controlled at 25.0 ± 0.1 °C by circulating water through cored cuvette holders from an external water bath. The protein solutions were placed in rectangular tandem cells (Hellma) of total path length of 0.878 cm divided equally with a quartz partition. In each cell, one compartment held 1.0 mL of an Fd′ solution and the other held an equal volume of a solution of the second reactant. All solutions were passed through a 0.45-μm Millipore filter immediately prior to each series of experiments. The concentration of Fd′ after mixture was held constant at 1.0×10^{-5} M[3] while that of the second species was varied from 10^{-6} to 4×10^{-5} M. In some experiments, Fd′ together with one of the κ-chain domains in molar excess was allowed to react to equilibrium in one compartment of each cell. The other domain was placed in the second compartment of each cell, and the contents of the sample cell were mixed. Prior to mixing a base line was established between 350 and 250 nm, and at zero time the contents of the sample cell were mixed by inversion. The change in absorbance, ΔA, at a constant wavelength (usually 292 or 301 nm) was recorded as a function of time, t, from a value of ΔA_0 at $t = 0$ to a plateau value ΔA_∞ at the end of the reaction. The equilibrium difference spectrum was then recorded. The reference cell was then mixed and a second set of kinetic data collected. The base line, spectrum, and kinetic data in digital form were collected on magnetic tape through a data logger prior to computer analysis. Several spectra for each class of recombination reaction were subsequently averaged. A nonlinear regression analysis of the kinetic data was performed as described in detail by Bunting et al. (1977) by using the following equation

$$\Delta A = \Delta A_\infty - \frac{([X]_0 - [Y]_0)(\Delta A_\infty - \Delta A_0)}{[X]_0 \exp[([X]_0 - [Y]_0)kt] - [Y]_0}$$

[3] Unless otherwise indicated, all protein concentrations are given as moles of monomer per liter.

where k is the second-order rate constant (M^{-1} s^{-1}) and $[X]_0$ and $[Y]_0$ are the initial concentrations of the reactants present in the higher and lower concentrations, respectively.

Analytical Ultracentrifugation. Sedimentation velocity and sedimentation equilibrium experiments were performed on a Beckman Model E analytical centrifuge at 20 °C on samples of Fd′, V_κ, and C_κ fragments in 4 mM sodium acetate buffer, pH 5.4. Ultraviolet absorption optics were used for the velocity experiments, and Rayleigh interference optics were used for the equilibrium runs.

Sedimentation coefficients were calculated from the change in radial position at the midpoint of the concentration plot as a function of time at 60 000 rpm. Linear regression was used to analyze these data by use of the usual relationship (Chervenka, 1970). At least three initial protein concentrations were used for each protein.

Equilibrium data were measured on a Nikon 6C Shadowgraph comparator modified so that the X–Y stage was driven by stepping motors. Fringe centers were located visually, and the coordinates, determined by pulse circuits driving the stepping motors, were recorded automatically on a digital cassette recorder. These data were transferred through a PDP-11 computer interface to an IBM-370 computer where it was analyzed by the program of Roark & Yphantis (1969). Plots of reciprocal point-average molecular weights vs. protein concentration (measured in millimeters of fringe displacement) and "ideal, two-species" plots were obtained on-line by a Gould electrostatic plotter. A value of 0.73 mL g^{-1} was assumed for the partial specific volume in all calculations.

NH_2-Terminal Sequence Determination. The V_κ-region subgroup determination for each of the κ chains used in this study was performed by sequence analysis in a Beckman Model 890C sequencer as described previously (Bunting et al., 1977). On the basis of the sequence of the first 10 amino acids, it is possible to assign κ chains to the $V_{\kappa I}$, $V_{\kappa II}$, or $V_{\kappa III}$ subgroup (Smith et al., 1971). The NH_2-terminal sequence of the C_κ fragments was also determined to assess the point of proteolytic cleavage. The desalted, freeze-dried samples (1–2 mg) were applied to the cup in 50% acetic acid. The PTH amino acids were identified by gas–liquid chromatography.

Radiolabeling of Proteins. V_κ fragments were labeled with carrier-free ^{125}I by using the lactoperoxidase method of Marchalonis (1969). In order to obtain ^{14}C-labeled C_κ fragments, reduced IgG was alkylated with [^{14}C]iodoacetamide (Amersham/Searle).

Electrophoresis and Immunological Techniques. Cellulose–acetate electrophoresis was performed in a Microzone cell (Beckman) at pH 8.6 by using barbital buffer (μ = 0.05). Sodium dodecyl sulfate–polyacrylamide electrophoresis was performed according to Maizel (1971) in 12% gels containing 0.1% sodium dodecyl sulfate. For molecular weight determinations, all samples were boiled in 2% sodium dodecyl sulfate at pH 8.0 for 2 min and then reduced with 20 mM DTE for 30 min at 37 °C and alkylated with 44 M iodoacetamide. A standard curve was obtained by plotting log molecular weight against the electrophoretic mobility of proteins of known molecular weight. Gels were stained overnight with Coomassie brilliant blue or Amido black. The micro method of Scheidegger (1955) was used for immunoelectrophoresis, and immunodiffusion analyses were performed in 1.5% agar (Agar Purified; Difco) in 0.15 M NaCl. Subclass typing of the IgG proteins was performed by double diffusion in agar by using monospecific antisubclass antisera. The V_H subgroup of the H chains and the K_m allotype of the

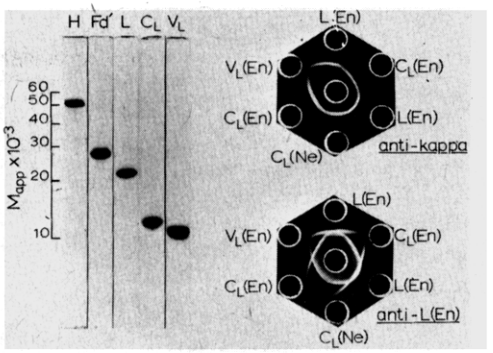

FIGURE 1: (Left) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of representative samples of the chains and fragments used in this study. Apparent molecular weights are given in Table I. (Right) Immunochemical characterization of the tryptic V_κ and C_κ fragments derived from κ chain (En) and the C_κ fragment from κ chain (Ne). The anti-κ antiserum used in the upper gel recognized antigenic determinants associated with the C_κ region only, whereas the antiserum raised against κ chain (En) recognized determinants in both the V_κ and C_κ regions.

κ chains were kindly determined by Dr. Liliane Rivat (Rouen, France) by passive hemagglutination. Antisera to the various κ chains used in this study were raised in rabbits and rendered specific for the V_κ or C_κ regions by appropriate absorptions.

Protein concentrations were determined spectrophotometrically at 280 nm by using the following extinction coefficients ($E_{1cm}^{1\%}$); κ chain, 12.5; Fd′, 13.0; C_κ, 14.0; and V_κ 10.0–12.0, depending on the fragment used.

Results

Characterization of Fragments. The κ chains from proteins En, Te, and Br were allocated to the $V_{\kappa III}$ subgroup by amino-terminal sequence analysis (Bunting et al., 1977) and shared the same Km3 allotype in the C_κ region. The V_H domain of the En-Fd′ fragment belonged to the V_{HI} subgroup.

The yields of fragments corresponding to the V_κ and C_κ domains obtained by peptic and tryptic cleavage of a number of mildly reduced and alkylated κ chains (including En, Te, and Br) varied depending on the κ chain and on the enzyme used. Three different situations were encountered. With some κ chains, a virtually theoretical yield of V_κ fragments was obtained but only negligible amounts of C_κ were obtained. In others, C_κ was recovered in good yield but no intact V_κ was obtained. Rarely, both domain fragments were generated although the yield of C_κ was always low. On cellulose–acetate electrophoresis at pH 8.6, the tryptic and peptic V_κ fragments from En, Te, and Br κ chains exhibited different mobilities but were all more basic than the parent κ chains, whereas the C_κ fragments migrated with similar anodic mobility.

Fd′ and the κ-chain fragments each gave a single band on $NaDodSO_4$–polyacrylamide gel analysis (Figure 1, left). The apparent molecular weights by this method were the following: Fd′, 28,300; C_κ, 11 500; and V_κ, 10 500. A single band was also observed in the presence of a reducing agent, indicating that there had been no significant secondary proteolysis within the polypeptide enclosed by the intradomain disulfide bond. Typical immunochemical analyses of the V_κ and C_κ fragments are shown in Figure 1 (right). Reactions of complete antigenic identity were obtained between the two C_κ fragments and the intact κ chain by use of an antiserum specific for the C_κ region (anti-κ). The V_κ and C_κ fragments gave reactions of partial identity with the intact κ chain and reactions of nonidentity with each other when tested by use of an antiserum raised against the autologous κ chain (i.e., an antiserum having anti-idiotype and anti-C_κ specificities). V_H and $C_\gamma 1$ antigenic

Table I: Hydrodynamic Properties of the Proteins Used in the Present Study[a]

protein	$s^0_{20,w}$	molecular weight: $NaDodSO_4$[b]	molecular weight: sedimentation equilibrium
κ chain			
monomer	2.4	22 500	23 000
dimer	3.6		46 000
V_κ fragment			
monomer	1.4	10 500	
dimer	2.3		22 000
C fragment			
monomer[c]	1.5	11 500	
Fd′ fragment			
monomer		28 000	28 600
dimer[d]			57 200

[a] See footnote 2. [b] By sodium dodecyl sulfate–polyacrylamide gel electrophoresis. [c] This fragment behaved as a monomer at all protein concentrations tested. [d] Higher oligomers of this fragment were observed by sedimentation equilibrium (see text and footnote 2).

determinants were demonstrated on isolated En-Fd′ by immunodiffusion against monospecific anti-idiotypic and anti-$C_\gamma 1$ antisera (data not shown). NH_2-terminal end-group analyses by dansylation (Gray, 1967) of the V_κ fragments in each case gave a glutamic acid corresponding to the NH_2-terminal residue of the parent κ chain. Automatic NH_2-terminal sequence analysis of the tryptic C_κ fragment showed that the enzyme had cleaved within the switch region between Arg-115 and Thr-116.

	115		120		125
κ chain:	-Lys-Arg-	Thr-Val-Ala-Ala-Pro-Ser-	Val-Phe-Ile-Phe-		
C_κ:		Thr-Val-Ala-Ala-Pro-Ser-	Val-Phe-Ile-Phe-		

The homogeneity of the dansylated NH_2-terminal residue of the V_κ fragments, on thin-layer chromatography, and the uniqueness of the C_κ sequence confirmed the absence of secondary cleavages. The integrity of the COOH terminus of the C_κ fragment was assessed by digesting a sample of the κ chain, which had been alkylated with [^{14}C]iodoacetamide, and by determining the molar content of [^{14}C]carboxymethylcysteine in the C_κ fragment.

Sedimentation velocity and equilibrium experiments were performed to determine the molecular dispersity of the fragments under the conditions used for recombination. The results are summarized in Table I. Intact κ chains and their isolated V-region fragments behaved as rapidly interconverting monomer–dimer mixtures in 4 mM sodium acetate buffer, pH 5.4, whereas C_κ fragments were monomeric over a comparable range of protein concentrations. The dispersity of the Fd′ fragments was more complex; they were found to associate indefinitely to form dimers, trimers, and higher species with an association constant of 6.2×10^3 M^{-1}.[4] At 0.3 mg mL^{-1}, the highest concentration of Fd′ after mixture used in the recombination studies, 80% of the fragments was in the monomeric form, 5% was dimeric, and the remainder was higher polymers.

Equilibrium Difference Spectra. At the initial protein concentration used in the spectroscopic experiments, more than

[4] The application of sedimentation equilibrium techniques to the determination of association constants and studies on the changes in circular dichroism accompanying interaction of the fragments used in the present studies will be described elsewhere (Klein, Kells, Kortan, and Dorrington, unpublished experiments).

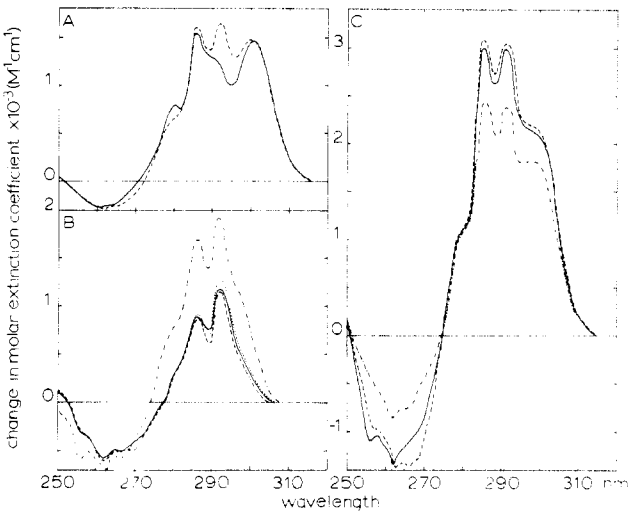

FIGURE 2: Difference spectra generated between 250 and 320 nm when Fd′ fragment (En) was recombined with intact κ chain or V_κ fragment or C_κ fragment in 4 mM sodium acetate buffer (pH 5.4) at 25 °C. (A) V_κ (En) recombined with Fd′ at a molar ratio of 2:1 (—). V_κ recombined with a preformed Fd′C_κ complex (- - -); the molar ratios V_κ/C_κ/Fd′ were 2:2:1. (B) Fd′ recombined with C_κ (En) (—) or C_κ (Ne) (- - -) or C_κ (Lu) (···). In each case the molar ratio C_κ/Fd′ was 2:1. C_κ (Lu) recombined with a preformed Fd′V_κ complex (-·-·); the molar ratios C_κ/V_κ/Fd′ were 2:2:1. (C) Fd′ recombined with intact κ-chain (En) (—); Fd′ reacted simultaneously with a twofold molar excess of both V_κ and C_κ fragments (- - -); calculated spectrum for Fd′ + κ chain obtained by addition of the spectra for Fd′ + V_κ and Fd′ + C_κ (-·-·-). The change in molar extinction coefficient was calculated as described under Materials and Methods.

80% of the κ chains and the Fd′ fragment were in the monomeric form. Between 70 and 90% of the V_κ fragment was in the monomeric form, immediately after mixture, depending on the protein concentration. No difference spectrum was observed when any of the fragments were diluted by mixing the sample cuvette in the absence of a second reactant. Similarly, when V_κ and C_κ fragments were mixed, there was no spectral evidence for an interaction between them.

A complete set of data for the recombination of Fd′ (En) with the intact κ chain and the V_κ and C_κ fragments, also from protein En, is shown in Figure 2. Each spectrum represents the average of 4–8 separate experiments. The interaction of V_κ with Fd′ yields a spectrum characterized by positive peaks at 282, 286, and 301 nm, by a shoulder at 292 nm, and by a shallow trough below 270 nm (Figure 2A). When V_κ was bound to a preformed binary complex between Fd′ and C_κ, the 292-nm peak, which was poorly defined in the V_κFd′ spectrum, was now well resolved (Figure 2A). Three different C_κ fragments (En, Lu, and Ne) each gave essentially the same difference spectrum when bound to Fd′ (Figure 2B). Resolved positive bands were apparent at 286 and 292 nm with evidence of another band centered near 295 nm, seen as a shoulder, and a progression of weak negative bands below 275 nm. The overall intensity of the difference spectrum above 275 nm was markedly increased when C_κ was bound to an Fd′–V_κ complex (Figure 2B). When intact κ chain is recombined with Fd′, the spectrum shown in Figure 2C is obtained. Intense positive bands were apparent at 286 and 292 nm, pronounced shoulders were apparent at 280 and 301 nm, and relatively weak negative bands were apparent below 270 nm. In order to determine to what extent this spectrum could be accounted for by the separate V_κ–V_H and C_κ–$C_\gamma 1$ interactions, a calculated recombination spectrum was obtained by using the following equation.

$$\Delta\epsilon_\lambda^{\text{calc}} = \Delta\epsilon_{\lambda_{Fd'+V_\kappa}}^{\text{expt}} + \Delta\epsilon_{\lambda_{Fd'+C_\kappa}}^{\text{expt}}$$

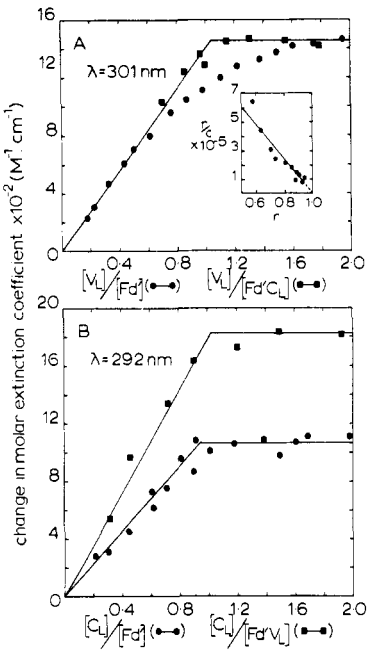

FIGURE 3: Equilibrium binding curves obtained from the difference spectra generated at different molar ratios of the interacting species. (A) V_κ + Fd′ (●) and V_κ + Fd′C_κ (■) recorded at 301 nm. The inset shows a Scatchard plot of the data for V_κ + Fd′ giving an association constant of 1.2×10^6 M^{-1}. (B) C_κ + Fd′ (●) and C_κ + Fd′V_κ (■) recorded at 292 nm. The final concentrations of Fd′, Fd′C_κ, and Fd′V_κ were held constant at approximately 1.0×10^{-5} M, and the concentrations of V_κ and C_κ were varied to give the molar ratios indicated. Values of the change in molar extinction coefficient were calculated from the concentration of the species present in the lower concentration as described under Materials and Methods.

Although the calculated spectrum showed the same qualitative features as that obtained experimentally for Fd′ and κ chain, the overall intensity of the spectrum was significantly lower (Figure 2C). In contrast, when V_κ and C_κ were recombined simultaneously with Fd′, the difference spectrum was quantitatively and qualitatively the same as that obtained with intact κ chain (Figure 2C).

Binding curves were constructed from equilibrium difference spectra obtained at different molar ratios of the interacting species (Figure 3). The final concentration of Fd′ was held constant, and the concentration of either V_κ or C_κ was varied systematically. The change in molar extinction coefficient ($\Delta\epsilon$), at the chosen wavelength, was calculated from the molar concentration of added κ-chain fragment when this concentration was lower than that of Fd′ and from the concentration of Fd′ when the molar ratios were greater than unity. Comparable binding curves were obtained when the concentrations of either V_κ or C_κ were held constant and [Fd′] was varied. As shown in Figure 3A, the value of $\Delta\epsilon$ at 301 nm increased as a function of [V_κ], reaching a plateau value at a molar ratio near 1.6. At intermediate molar ratios it was possible to determine concentrations of bound and free V_κ which, when subjected to Scatchard (1949) analysis, gave an intrinsic association constant of 1.2×10^6 M^{-1}. In a second series of experiments, V_κ was allowed to interact with a preformed complex of Fd′ and C_L (Figure 3A). $\Delta\epsilon_{301nm}$ increased as a linear function of [V_κ] with a sharp inflection occurring at an equimolar ratio of reactants, indicating that the presence of C_κ enhances the affinity of Fd′ for V_κ. The binding curve obtained for the interaction of C_κ with Fd′ also showed a sharp inflection at equimolar concentrations, so it was not possible to calculate the association constant (Figure 3B). Although the shape of the binding curve for C_κ interaction with Fd′–V_κ was the same as that obtained with Fd′ alone, the plateau value of $\Delta\epsilon_{292nm}$ was almost twice as great (1820 vs. 1050 M^{-1} cm^{-1}).

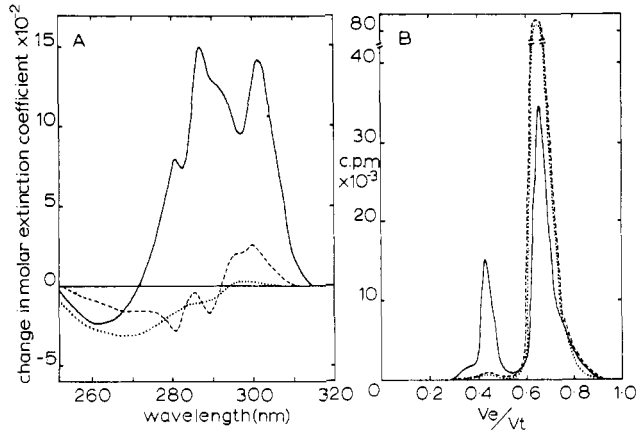

FIGURE 4: (A) Difference spectra observed when Fd′ (En) was recombined with autologous V_κ (—) and with two heterologous V_κ fragments, V_κ (Te) (- - -) and V_κ (Br) (···) in 4 mM sodium acetate (pH 5.4) at 25 °C. (B) In separate experiments γ-chain dimer (En) was mixed with a fourfold molar excess of [^{125}I]V_κ (En) or [^{125}I]V_κ (Te) or [^{125}I]V_κ (Br) and chromatographed on a column of Sephadex G-150 equilibrated in 0.1 M NaCl, 20 mM sodium acetate buffer (pH 5.4). Only in the case of the autologous recombinant (—) was the expected 25% of the radioactivity recovered at the elution position of a $\gamma_2V_{\kappa_2}$ species. With V_κ (Te) (- - -) and V_κ (Br) (···) virtually all of the radioactivity was recovered as V_κ dimer.

The data given in Figure 1B clearly showed that the features of the spectra obtained when Fd′ (En) reacted with three different C_κ fragments were very similar. This was to be expected since the fragments were chemically identical. At the V_κ-region level some spectral differences might have been expected due to differences in the hypervariable sequences and, to a lesser extent, in the framework. When we attempted to test this experimentally, the results shown in Figure 4A were obtained. If Fd′ (En) was interacted with V_κ (En), the expected difference spectrum was obtained; however, two heterologous V_κ fragments (Te and Br) gave spectra of very low intensity even at high molar ratios. Two possible explanations for these observations were considered: either the heterologous V_κ regions have little or no affinity for V_H or they do bind, in fact, but the enviroments of aromatic chromophores are not perturbed. To distinguish between these two possibilities, the three V_κ fragments were labeled with ^{125}I and separately mixed in fourfold molar excess with En-γ chains.[5] After standing overnight, to ensure equilibrium had been obtained, the mixtures were passed over a calibrated column of Sephadex G-150 equilibrated with 0.1 M NaCl and 20 mM sodium acetate buffer, pH 5.4, and the distribution of radioactivity in the effluent was determined (Figure 4B). Only in the autologous mixture, En-V_κ + En-γ chains, was the expected proportion of the counts (i.e., 25%) eluted at a $V_e/V_t = 0.42$, consistent with the recombinant, $\gamma_2V_{\kappa 2}$. In the heterologous mixtures virtually all the radioactivity was eluted at a position corresponding to the free V_κ fragments. These results showed that the difference spectral data was accounted for by the inability of Te- and Br-V_κ fragments to interact with En-Fd′. Exactly comparable data were obtained when two other Fd′ fragments were mixed with autologous and heterologous V_κ

[5] γ-Chain dimers were chosen for these studies to maximize the molecular size difference between the recombinant and the free V_κ, a substantial proportion of which was in the dimeric form at the protein concentrations used. Preliminary studies had shown that γ chains recombined with both V_κ and C_κ in a fashion identical with that of Fd′.

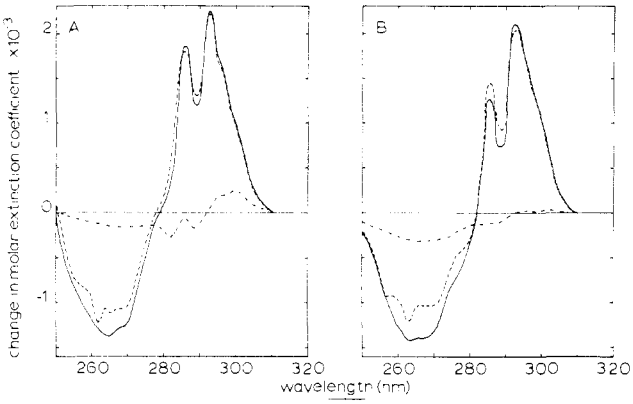

FIGURE 5: (A) Difference spectra observed when Fd′ (En) was recombined with a twofold molar excess of V_κ (Te) (····) or simultaneously with a twofold molar excess of both V_κ (Te) and C_κ fragments (- - -) or with the intact κ chain (Te) (—). (B) Spectra obtained when Fd′ (En) was mixed with a twofold molar excess of V_κ (Br) (····) or with a twofold molar excess of both V_κ (Br) and C_κ fragments (- - -) or with the intact κ chain (Br) (—).

Table II: Second-Order Forward Rate Constants for the Recombination of κ Chain and Its Fragments with Fd′

recombination	$k_{12}^{26\ °C}$ (M^{-1} s^{-1})[a]
Fd′ + κ	206 ± 31 (16)
Fd′ + V_κ	202 ± 19 (7)
Fd′C_κ + V_κ	213 ± 12 (14)
Fd′ + C_κ	12 ± 2 (5)
Fd′V_κ + C_κ	77 ± 10 (10)

[a] Rate constants determined by nonlinear regression analysis are given as mean ± SEM. The number of determinations is shown in parentheses.

fragments. Previous studies had shown, however, that heterologous recombinations by use of intact κ chains occur readily (Bunting et al., 1977). We decided, therefore, to reevaluate the recombination of heterologous V_κ fragments in the presence of C_κ. When En-Fd′ was reacted simultaneously with either Te-V_κ or Br-V_κ in the presence of C_κ, difference spectra were obtained which were essentially identical with those obtained when the intact κ chains were mixed with En-Fd′ (Figure 5A and B). Thus, in the presence of C_κ, effective union between V_H and heterologous V_κ fragments takes place.

Kinetic Studies. The time course of the appearance of the difference spectrum was followed at 301 and 292 nm for reactions involving V_κ and C_κ fragments, respectively. Representative data for autologous reactions are shown in Figure 6. The kinetics of recombination for both κ-chain fragments were shown to be second order (Figure 6 and Table II). The second-order forward rate constant governing the binding of V_κ to Fd′ was not significantly different from that observed for the binding of intact κ chain, and it was not influenced by the presence of C_κ. The rate constant for the interaction of C_κ with Fd′ was only one-sixth of that for the Fd′–V_κ interaction but was significantly increased in the presence of V_κ (Figure 6B).

Discussion

The objectives of the present study were threefold: firstly, to evaluate the relative importance of V_L–V_H and C_L–$C_\gamma 1$ interactions in the binding of the L chain to Fd′; secondly, to determine if the prescence of one L-chain domain influenced the binding of the second; and, finally, to determine if there were any preferential interactions between autologous V_L and V_H at the level of isolated domains. To minimize structural differences, κ chains were selected that have identical C_κ-region

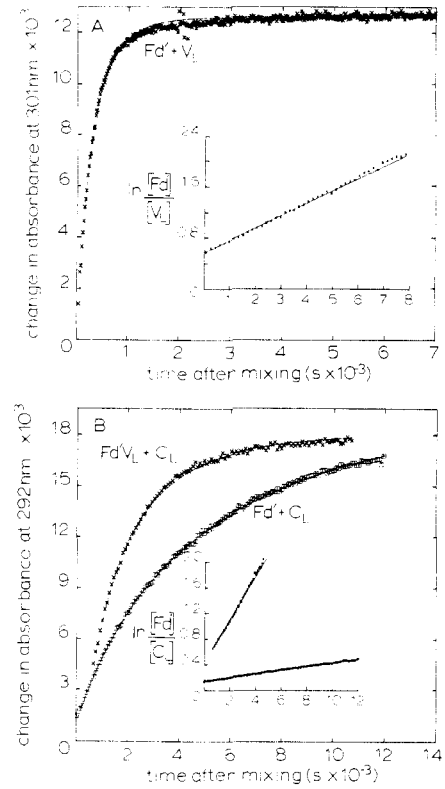

FIGURE 6: Representative kinetic data for the interaction of Fd′ with V_κ (A), Fd′ with C_κ (B), and Fd′V_κ with C_κ (B). The changes in absorbance, at the indicated wavelenths, as a function of time were recorded directly from the digital output of the spectrophotometer onto magnetic tape. The line through the data points was derived from the theoretical second-order equation (eq 1 under Materials and Methods). Conditions: (A) [Fd′] = 7 × 10^{-6} M, [V_κ] = 12 × 10^{-6} M; (B) [Fd′] = 10 × 10^{-6} M, [C_κ] = 9 × 10^{-6} M; [Fd′V_L] = 6.5 × 10^{-6} M, [C_κ] = 10 × 10^{-6} M. The insets show the data plotted by using the more usual, logarithmic form of eq 1. The following values for k_{12} were obtained from the slopes: Fd′ + V_κ, 209 M^{-1} s^{-1}; Fd′ + C_κ, 12 M^{-1} s^{-1}; Fd′V_κ + C_κ, 77 M^{-1} s^{-1}.

sequences and belong to the same V_κ-region subgroup.

Characteristics of Fragments. Sedimentation velocity and equilibrium studies showed that the $V_{\kappa III}$ fragments, produced by peptic and tryptic cleavage, were in monomer–dimer equilibrium with a dimerization constant near 10^5 M^{-1} (Klein et al., unpublished experiments). Similar values have been obtained for a $V_{\kappa I}$ fragment (Maeda et al., 1976) and a V_λ fragment (Azuma et al., 1978). The magnitude of the dimerization constant for V_κ regions is comparable to that governing intact L-chain dimerization, i.e., 10^4–10^5 M^{-1} (Green, 1973; Azuma et al., 1976; Klein et al., 1977). In contrast, the C_κ fragments were monomeric over a wide range of concentrations. Identical findings have been reported for other C_κ—as well as C_λ—fragments (Solomon & McLaughlin, 1969; Karlsson et al., 1972; Seon et al., 1972; Azuma et al., 1978). These observations suggest that the dimerization of native L chains is probably initiated by a V_L–V_L interaction which brings the C domains into close apposition, allowing hydrophobic interaction between their four-chain β sheets (Schiffer et al., 1973). This hypothesis is supported by the observations of Stevenson & Straus (1968) who found specific dimerization of autologous human L chains even in the presence of heterologous subunits.

Despite attempts to minimize the structural differences between the κ chains used, their susceptibility to proteolysis, as judged by the yields of V_κ and C_κ fragments, showed marked variation. Since the percentage of κ-chain monomers present at the molar concentrations used for proteolysis could have

ranged from 0.2 to 50% (by assuming that the individual dimerization constants lay in the range 10^4–10^5 M^{-1}), the absolute and relative yields of the fragments may have reflected the relative susceptibility of the monomer and dimer and their proportions in the original sample.[6] In addition, the sequence and tertiary folding of the hypervariable segments may affect the intrinsic susceptibility of individual V_κ regions as well as the degree to which the switch region is exposed to enzymic attack. Special care was taken in the present study to ensure that the fragments used had no secondary cleavages within the domains. The V_κ and C_κ fragments retained the antigenic determinants exhibited by the intact κ chain and had secondary and tertiary structures consistent with them, being in the native conformation as judged by circular dichroism (data to be published). The lack of any major perturbation of the V-region conformation upon removal of the C region is supported by the X-ray crystallographic studies of Colman et al. (1977). These workers studied three $V_{\kappa I}$ dimers and found that the folding of the polypeptide backbone and the quaternary relationship between the two monomers appeared to be the same as the Mcg λ-chain dimer (Epp et al., 1974; Schiffer et al., 1973).

(*1*) *Autologous Recombinations.* Isolated V_κ and C_κ domains each formed a 1:1 complex with either the Fd′ fragment or the preformed binary complex between Fd′ and the complementary domain fragment. These data demonstrate a high degree of specificity between V_κ and V_H and between C_κ and $C_\gamma 1$, excluding significant degeneracy, i.e., V_κ–$C_\gamma 1$ and C_κ–V_H interactions. These results confirm and extend those of Smith & Dorrington (1972) who showed by gel chromatography that V_κ and C_λ fragments reassociated with γ chains and those of Levo et al. (1977) who interacted a κ chain with a μ chain lacking the V_H region and with a $C_\gamma 1$ fragment isolated from a γ_3 chain. The difference spectra generated upon recombination of Fd′ with V_κ and C_κ indicate that aromatic chromophores, exposed to solvent in the free subunits, are transferred to a less polar environment upon recombination (Donovan, 1969). The positive difference peak at 292 nm can be unambiguously assigned to the 0–0^1L_b electronic transition of the indole chromophore. On the basis of studies with model compounds, the burial of a fully exposed indole ring would be expected to give a $\Delta\epsilon_{292nm}$ near 1600 M^{-1} cm^{-1} (Donovan, 1969) so that the equivalent of 0.8 and 0.75 tryptophanyl side chains was buried when Fd′ bound V_κ and C_κ, respectively. The magnitude of the positive peak at 286 nm is highly suggestive of the burial of tyrosyl phenolic groups. The peak at 301 nm, characteristic of the V_κ–V_H association, probably reflects a charge perturbation of a tryptophanyl transition (Ananthanarayanan & Bigelow, 1969a,b). Involvement of phenylalanine is suggested by the characteristic series of weak bands between 255 and 270 nm. Recent high-resolution X-ray crystallographic analyses together with sequence data on a human Fab′λ (NEW), a λ-type Bence-Jones protein (Mcg), and a $V_{\kappa I}$ dimer (REI) indicate that several conserved aromatic residues are present in the contact region between V_H and V_L: Tyr-36 on all V_L domains and Trp-47, Tyr-95, and Trp-108 on V_H (NEW) (Davies et al., 1975a,b). From available sequence data, identical residues can be assigned to similar positions in the $V_{\kappa III}$ and V_{HI} domains used in the present studies. C_κ and C_λ domains are similar in structure, and aromatic residues found within the intersubunit contact region of C_L, such as Phe-139, Trp-148, and Tyr-192, are common to both C_κ and C_λ. This chemical information, therefore, supports the interpretation of the spectral data.

The interactions of Fd′ with L chain and the isolated V and C regions may be summarized as

$$Fd'_2 \rightleftharpoons 2Fd' \quad (K_A \sim 10^3\ M^{-1}) \tag{2}$$

$$L_2 \rightleftharpoons 2L \quad (K_A \sim 10^4\text{–}10^5\ M^{-1}) \tag{3}$$

$$Fd' + L \rightleftharpoons Fd'L^* \rightleftharpoons Fd'L\ (\equiv Fab') \quad (K_A > 10^{10}\ M^{-1}) \tag{4}$$

$$V_2 \rightleftharpoons V_2^* \rightleftharpoons 2V \quad (K_A \sim 10^5\ M^{-1}) \tag{5}$$

$$Fd' + V \rightleftharpoons Fd'V \quad (K_A \sim 10^6\ M^{-1}) \tag{6}$$

$$Fd' + C \rightleftharpoons Fd'C \quad (nd) \tag{7}$$

Reactions 2–4 have been previously discussed by Bigelow et al. (1974). Circular dichroism studies of the reassociation of a tryptic Fd′ fragment with a κ chain led Azuma & Hamaguchi (1976) to postulate that reaction 4 involved the formation of an intermediate, FdL*, followed by a unimolecular rearrangement. Temperature-jump kinetic studies on the dimerization of a $V_{\kappa I}$ fragment showed the presence of two isomers of the dimer in equilibrium at a ratio of approximately 1:1 (Maeda et al., 1976). Since the equilibrium constant governing the dimerization of V_κ is an order of magnitude lower than that governing the reassociation of Fd′ with V_κ, reaction 6 can go to completion in the presence of a molar excess of V_κ.

Reactions 6 and 7 both followed second-order kinetics, indicating that the difference spectra resulted from subunit interaction per se and not from isomerization of the recombinant. A conformational change would have imposed first-order kinetics on the overall reaction.[7] There were marked differences in the rates at which V_κ and C_κ were bound to Fd′ (200 and 12 M^{-1} s^{-1}, respectively) with the rate of the Fd′ + V_κ reaction being the same as that for the interaction of the intact κ chain with Fd′ (206 M^{-1} s^{-1}). V_κ–V_H interactions, therefore, dominate the kinetics of the Fd′–κ chain recombination. In terms of the overall free energy changes for reactions 6 and 7, however, it is clear that the high-affinity interaction between Fd′ and L ($K_A > 10^{10}$ M^{-1}) derives from relatively weak interactions between V_H and V_L and between $C_\gamma 1$ and C_L ($K_A \sim 10^6$ and $> 10^6$ M^{-1}, respectively). When V_L and C_L are covalently linked the overall "avidity" of L chain for Fd′ (or γ chain) could obviously become very large (i.e., $>10^{12}$ M^{-1}).

The experiments designed to evaluate the influence of one domain fragment on the binding characteristics of the second fragment yielded several interesting results. The affinity of V_κ for V_H was enhanced when C_κ had already been bound to the $C_\gamma 1$ region of Fd′ although the kinetics of binding were unaffected. The magnitude of the $\Delta\epsilon_{292nm}$ peak for V_κ + Fd′C_κ suggested that 1 equiv of tryptophan was buried compared with 0.8 equiv in the absence of C_κ. A more marked increase in spectral intensity was observed for the binding of C_κ in the presence of V_κ together with a sixfold increase in the rate at which C_κ was bound (77 vs. 12 M^{-1} s^{-1}). There are two possible explanations for these phenomena: (1) the binding of one κ-chain domain, for example, V_κ to V_H, modulates the

[6] Earlier studies showed that disulfide-bonded L-chain dimers were relatively resistant to proteolysis but became susceptible following mild reduction (Solomon & McLaughlin, 1969).

[7] Slight deviations from second-order behavior was observed in some recombinations of Fd′ with C_κ at long reaction times. It has not been possible to determine whether these have any mechanistic significances or whether they are due to an experimental artifact due to the long times over which these slow reactions had to be followed.

conformation of the adjacent $C_\gamma1$ region, thus altering its reactivity toward C_κ, or (2) contacts are made between the bound and the incoming fragment which, of course, would not be apparent when Fd′ reacts with either fragment separately. The observation that the difference spectrum generated when V_κ and C_κ fragments were interacted simultaneously with Fd′ was essentially identical with that observed for Fd′ + L argues against the second possibility. The other possibility will be discussed below. The ternary complex, Fd′$V_\kappa C_\kappa$, was very similar to the parent Fab′ by several criteria: it had the same molecular weight as judged by gel filtration, showed full antigenic identity with Fab′ by use of several different antisera, and gave similar circular dichroic spectra (unpublished experiments).

(2) *Heterologous Recombinants.* Perhaps the most striking finding of these studies was the failure of heterologous $V_{\kappa III}$ fragments to bind to Fd′ unless C_κ was present. When either Te-V_κ or Br-V_κ was bound simultaneously with C_κ to En-Fd′, the spectra obtained were virtually superimposable on those observed for En-Fd and the intact Te- and Br-κ chains. These data suggest that the trans interaction between C_κ and $C_\gamma1$ (which, as expected, shows no preference for autologous domains) induces a conformational change in the V_H region. This putative change may be transmitted through the longitudinal contacts which have been shown to exist between $C_\gamma1$ and V_H in Fab fragments and monomer 1 of the Mcg Bence-Jones dimer (Davies et al., 1975a,b; Huber et al., 1976). Reactions 6 and 7 can, therefore, be modified and extended as

$$\text{Fd}' + \text{V} \rightleftharpoons \text{Fd}\cdot\text{V}^* \rightleftharpoons \text{Fd}'\cdot\text{V} \quad (8)$$

$$\text{Fd}'\cdot\text{V} + \text{C} \rightleftharpoons \text{Fd}'\cdot\text{V}\cdot\text{C}\ (\equiv\text{Fab}') \quad (9)$$

$$\text{Fd}' + \text{C} \rightleftharpoons \text{Fd}\cdot\text{C}^* \rightleftharpoons \text{FdC} \quad (10)$$

$$\text{Fd}\cdot\text{C} + \text{V} \rightleftharpoons \text{Fd}\cdot\text{C}\cdot\text{V}\ (\equiv\text{Fab}') \quad (11)$$

Preferential recombination between autologous γ and L chains has been repeatedly observed in competitive hybridization experiments in which γ_A is reacted with a mixture of a radiolabeled autologous L chain ($[^{125}I]L_A$) and a heterologous L chain ($[^{131}I]L_B$) (Grey & Mannik, 1965; Mannik, 1967; Gergerly et al., 1973; Stevenson & Mole, 1974; de Preval & Fougereau, 1976). Bunting et al. (1977) were unable to find evidence that differences in the rate of autologous vs. heterologous reactions could explain this phenomenon even for chains which showed preferential recombination under equilibrium conditions. In the present study, specific recognition between V_H and $V_{\kappa III}$ regions was observed for three pairs of autologous Fd′ and V_κ fragments. These findings suggest that complementarity-determining regions play a role in the specific interaction between V regions. Interactions between the hypervariable regions of V_L and V_H have been observed in crystals of Fab fragments and of the Mcg Bence-Jones dimer (Davies et al., 1975a,b). Another possibility is that amino acid substitutions in the framework of the V regions, perhaps together with differences in hypervariable sequences, may induce subtle changes in the V_H–V_L interface. The experiments of de Preval & Fougereau (1976) argue against the suggestion that preferential recombination may be due to "hysteresis"; i.e., chains which have been folded together retain some "memory" of this association which promotes their subsequent recombination. These workers showed that preferential interaction was still apparent when the separated γ and L chains were reduced and completely denatured and subsequently refolded and reoxidized. In our experiments, the presence of the C_κ region apparently abolished the specificity of the V_H–V_κ interaction. These observations suggest that the C_κ–$C_\gamma1$ interaction results in some conformational change in the V_H region which overcomes the putative structural constraints upon degenerate V_κ–V_H association. These data might suggest that C_L–C_H interactions serve to compensate for limitations on the extent of random V_κ–V_H association. However, preferential association may also be important at the intracellular level as indicated by the experiments of Margulies et al. (1976). They analyzed subclones of mouse hybrid cells synthesizing an IgM_λ and an $IgG1_\kappa$ and found that the λ chain associated less well with the heterologous γ_1 chain in vivo. If the preferential recombination we observed in vitro has any biological significance under normal conditions (remembering that the solvent conditions obtained in vitro and in vivo are somewhat different), then it creates problems since some linkage between the expression of particular γ and L chains appears to be required. The genes coding for the two chains are present on different chromosomes, so some novel type of transcription control would seem to be required. Another possibility, of course, is that the difference in the association constants governing autologous and heterologous recombination is so small [estimated to be about 1 order of magnitude by Stevenson & Mole (1974)] as to have little significance in vivo, particularly when, by definition, only autologous association is taking place.

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Isolation and Characterization of Detergent-Solubilized Human HLA-DR Transplantation Antigens†

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ABSTRACT: HLA-DR antigens have been isolated from spleens, in vitro grown Daudi cells, and leukocytes obtained from patients suffering from chronic lymphatic leukemia. Highly purified detergent-solubilized HLA-DR antigens were obtained by affinity chromatography on a *Lens culinaris* lectin column, two gel chromatographies, immunosorbent purifications, and diethylaminoethyl-Sephadex chromatography. In the gel chromatography steps the HLA-DR antigens separated into two fractions. The material of the larger size, fraction I, was converted to the smaller form, fraction II, on storage. The two HLA-DR antigen fractions, I and II, were each shown to be highly purified by chemical, physical–chemical, and immunological criteria. Material in both fractions appeared equally reactive with xenoantisera and alloantisera, suggesting that antigenic differences did not account for the size separation. HLA-DR antigens in fractions I and II displayed identical profiles on isoelectric focusing, and both were heterogeneous with regard to charge. Molecular weight determinations by gel chromatography in 6 M guanidine hydrochloride and by sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicated that the HLA-DR antigens in fractions I and II were composed of two types of polypeptide chains with the apparent molecular weights 28 000 and 34 000. The two HLA-DR antigen subunits could be dissociated and separated by isoelectric focusing in 9 M urea. The separated chains were both shown to bind detergents in micellar form as revealed by charge shift electrophoreses and gel chromatography separations. This result strongly suggests that both HLA-DR antigen chains are integrated into the hydrocarbon matrix of the cell membrane. Some physical–chemical properties of the isolated HLA-DR antigens in fractions I and II were determined.

The human major histocompatibility complex (MHC)[1] consists of at least four loci that code for cell surface molecules involved in several immunological reactions (for a review, see Thorsby, 1974). Three loci, HLA-A, -B, and -C, control the expression of the classical transplantation antigens. The HLA-D locus, primarily defined as the main locus responsible for the stimulation in the mixed leukocyte culture reaction, also controls the expression of cell surface antigens (Wernet et al., 1975). The HLA-D locus is believed to be the human counterpart to the better defined murine H-2 Ir region. Thus,

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[1] Abbreviations used: MHC, major histocompatibility complex; CTAB, cetyltrimethylammonium bromide; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; TDGH, tartryl bis(glycylhydrazide); DTT, dithiothreitol.

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