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Chemical Properties of Functional Groups of Mouse Immunoglobulins of the IgA, IgG2a, and IgM Classes†

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ABSTRACT: The chemical properties (p*K* and reactivity) of the histidine, lysine, tyrosine, cysteine, and N-terminal residues of three mouse myeloma proteins, MOPC 167, UPC 10, and MOPC 104E, representing the three major immunoglobulin classes IgA, IgG, and IgM, were determined by the method of competitive labeling [Kaplan, H., Stevenson, K. J., & Hartley, B. S. (1971) *Biochem. J. 124*, 289–299] using radiolabeled 1-fluoro-2,4-dinitrobenzene. The properties of the N-terminal residues showed relatively small individual variations, but all were in accord with these groups being completely exposed to solvent as X-ray crystallographic studies on other immunoglobulins have shown. The histidine residues of the three immunoglobulins had a narrow range of average p*K* values (7.2–7.3), but the histidines of the IgG had a much lower overall reactivity than those of the IgA or IgM. The reactivities of the latter two were greatly enhanced over the reactivity of a free imidazole group, indicating interactions of these residues with other elements of the proteins' structures. The lysine residues showed considerable variation in average p*K* values (9.7–10.4) and some variation in reactivity. In contrast, the tyrosine residues of the three immunoglobulins were all similar in behavior. These observations are consistent with the partial sequence data available for the three Ig's as the tyrosine residues show a greater degree of homology in the sequences than the lysine residues. The cysteine residues of the IgA had an apparent p*K* of 9.22 and a reactivity which indicated that at least some of the cysteines were exposed to solvent. In contrast, the small amount of cysteine detected in the IgM preparations had a much lower p*K* (8.5) and reactivity, suggesting less exposure to solvent. The overall behavior of the various groups examined demonstrated that the method of competitive labeling is well suited to immunoglobulin studies and when applied to individual groups in the proteins should provide many internal probes for studying the effects of antigen on antibody structure, which may be relatable to biological properties.

It is not clear how encounters with antigen cause antibodies to initiate the various physiological processes they control. Three theories have been considered (Metzger, 1974, 1978). The first is an allosteric one in which binding of an antigen by the Fab region of an antibody leads to a conformational change in the Fc region. There is evidence favoring this mechanism for the activation by IgM antibodies of the complement system (Brown & Koshland, 1975, 1977; Chiang & Koshland, 1979a,b). The second theory is an associative one in which antigen causes juxtapositioning of antibody molecules, and the resulting aggregate, by virtue of the multivalence of its multiple Fc sites, has novel properties. For example, IgE oligomers but not monomers can cause degranulation of mast cells, releasing effector molecules such as histamine (Segal et al., 1977). The third theory is a variation of strain theory in which occupation of the binding sites by bulky or multivalent antigens causes distortion at the hinge region, hence altering the Fc region. At present, there is no reason to assume that all the classes of antibody act by identical mechanisms nor are the three theories mutually exclusive.

Discrimination among these theories may be possible by examining the properties of particular functional groups in antibodies, in the presence and absence of antigen. Changes throughout the antibody molecules could be taken as evidence for an allosteric theory whereas lack of change would favor an associative theory. Most work of this nature has centered on the spectroscopic properties of tyrosine and tryptophan residues [reviewed by Metzger (1974, 1978)], and the technique of circular polarization of luminescence has given some evidence for conformational changes in the Fc region of IgG accompanying antigen binding (Jaton et al., 1975; Pecht et al., 1977) but without a convincing correlation with biological properties (Metzger, 1978). On the other hand, chemical and proteolytic studies of the hinge region of IgG have indicated that no changes were caused by antigen (Wright et al., 1978).

The present study used the competitive labeling approach (Kaplan et al., 1971) with a view to elucidating the chemical properties of functional groups in immunoglobulins representing the three major antibody classes, with varying biological properties. Knowledge of the properties of the native molecules indicates their structural characteristics and is an essential prerequisite for studies of alterations which may occur

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on antigen binding. The labeling technique employed was a development of methods described previously (Cruickshank & Kaplan, 1975; Duggleby & Kaplan, 1975) and used [^{3}H]- and [^{14}C]fluorodinitrobenzene to determine the average chemical properties (reactivity and pK) of lysine, tyrosine, histidine, and cysteine residues in the immunoglobulins and the properties of their unique N-terminal residues.

The immunoglobulins chosen were mouse myeloma proteins of known antigen specificity, for which there are considerable sequence data available. The complete sequence of both the light and heavy chains of the dextran-specific (Leon et al., 1970) MOPC 104E IgM(λ) are known (Appella, 1971; Kehry et al., 1979). For the phosphorylcholine-specific (Leon & Young, 1971) MOPC 167 IgA(κ), sequence data are available for almost all of the Fab region (Gray et al., 1967; Rudikoff & Potter, 1976, 1978; Francis et al., 1974; Robinson & Appella, 1977) and the C-terminal sequence of the heavy chain (Francis et al., 1974). Only the composition of the J chain in these two immunoglobulins is known (Barger & Inman, 1976), though homology to the human sequence (Mole et al., 1977) is to be expected. For the levan-specific (Cisar et al., 1974; Weigert et al., 1974) UPC 10 IgG2a(κ), the sequences of the four constant domains are known from other proteins (Gray et al., 1967; Bourgois et al., 1974) and from the N-terminal sequence of the light chain (Potter et al., 1977).

Materials and Methods

Immunoglobulins. Sera were obtained from BALB/c mice bearing the plasmacytoma lines MOPC 167 and MOPC 104E, propagated from stock provided by Dr. M. Potter. Freeze-dried UPC 10 ascites fluid was purchased from Litton Bionetics (Kensington, MD). The MOPC 104E IgM was purified by using the dextran affinity medium of Hiramoto et al. (1972); the protein was eluted with 1 M methyl α-D-glucoside. The MOPC 167 IgA was purified in the dimer form by the affinity chromatography method of Chesebro & Metzger (1972) with the reduction step being omitted. The UPC 10 IgG2a was purified by affinity chromatography on a staphylococcal protein A–Sepharose column (Pharmacia Ltd., Montreal) as described by Ey et al. (1978), followed by gel filtration on Sephadex G-200. Purity of the proteins was established by acrylamide and sodium dodecyl sulfate gel electrophoreses and immunodiffusion tests with appropriate antisera.

Chemicals. Unlabeled Dnp-F[1] was purchased from Aldrich Chemical Co., [^{3}H]- and [^{14}C]Dnp-F and [^{14}C]iodoacetic acid were from Amersham Corp., and Porapak Q was from Waters Associates Inc. β-L-Imidazolelactic acid (referred to hereafter as imidizolelactate) was purchased from Sigma Chemical Co., and Dnp-imidazolelactate was prepared from it as previously described (Duggleby & Kaplan, 1975). Dnp-glutamic acid was purchased from Sigma Chemical Co., and *S*-Dnp-cysteine was synthesized by reacting *N*-acetylcysteine with Dnp-F, followed by hydrolysis with 6 N HCl and isolation by high-voltage paper electrophoresis. The compound, detected by its ultraviolet absorption, was eluted with 0.01 M HCl and freeze-dried.

Protein Analyses. Amino acid compositions were obtained by analysis of 20-h digests on a Durrum D500 amino acid analyzer. Thiol contents were estimated by incorporation of [^{14}C]iodoacetic acid into the proteins in 3 M guanidine hydrochloride, pH 7.2, at room temperature.

Trace Labeling. A 2-mL sample of the immunoglobulin MOPC 167 IgA (0.77 mg/mL) in 5 mM *N*-methylmorpholine, 5 mM sodium borate, and 0.10 N KCl containing 20 nmol of imidazolelactate (the internal standard) was temperature equilibrated at 20 °C in a vessel with a thermostated water jacket. The pH was adjusted to the desired value with 1 N KOH, and 25 μL of acetonitrile containing 12.3 nmol of [^{3}H]Dnp-F (sp act. 14 Ci/mmol) was added with rapid stirring. The reaction mixture was transferred to a stoppered test tube and incubated at 20 °C for 18 h followed by the addition of 1 drop of concentrated HCl. Identical procedures were carried out for MOPC 104E IgM (1.37 mg/mL) and UPC 10 IgG2A (0.38 mg/mL).

[^{14}C]Dinitrophenylation. The proteins were made chemically homogeneous by treatment with an excess of [^{14}C]Dnp-F (25 μL of Dnp-F, 40% in acetonitrile, containing 18 μCi of [^{14}C]Dnp-F) as previously described (Duggleby & Kaplan, 1975).

Purification of Dnp-imidazolelactate (Internal Standard). Solutions of Dnp-albumin (5 mg) and Dnp-imidazolelactate (0.6 mg) were added as carriers, and each reaction mixture was acidified with concentrated HCl. One drop of octanol was added to prevent foaming. The reaction mixtures were extracted several times with diethyl ether until no further dinitrophenol remained and then were centrifuged to separate the precipitated Dnp-protein. The supernatants containing the Dnp-imidazolelactate were adsorbed on Porapak Q columns formed in Pasteur pipets and equilibrated with 0.01 M HCl. The columns were washed with 5 mL of 0.01 M HCl, and then each of the internal standards was eluted with 3 mL of 20% (v/v) acetone–water. The final purification was carried out by high-voltage paper electrophoresis at pH 3.5 and 2.1. The Dnp-imidazolelactate was located by its ultraviolet absorption.

Isolation of Dnp-aspartic and Dnp-glutamic Acids. The Dnp-proteins were hydrolyzed in 1-mL aliquots of 6 M HCl for 17 h, and the hydrolysates were extracted with 3 volumes of ether. The extracts were evaporated to dryness, carrier Dnp-glutamic acid was added, and the Dnp-aspartic (carrier being provided by the N terminal of Dnp-albumin) and Dnp-glutamic acids were separated on silica plates by two-dimensional descending chromatography using toluene–pyridine–2-chloroethanol–0.8 M ammonia (10:1:2:2 v/v, upper phase) in the first dimension and benzene–pyridine–acetic acid (4:1:1 v/v) in the second dimension. Removal of the spots was effected by scraping, and the compounds were eluted with 80% aqueous acetone.

Isolation of S-Dnp-cysteine, O-Dnp-tyrosine, ϵ-Dnp-lysine, and Imidazolyl-Dnp-histidine. Carrier *S*-Dnp-cysteine was added to the aqueous phase from the ether-extracted protein hydrolysates. The samples were dried under vacuum, dissolved in pH 2.1 buffer (acetic acid–formic acid–water, 4:1:45), spotted on Whatman 3MM paper, and subjected to high-voltage electrophoresis for 90 min at 3 kV. The mobilities relative to ϵ-Dnp-lysine were the following: *S*-Dnp-cysteine, 0.30; *O*-Dnp-tyrosine, 0.84; imidazolyl-Dnp-histidine, 1.85. The various components were located by their ultraviolet absorption and eluted from the paper with 0.01 M HCl.

Radioactivity Measurements and Calculations. All samples were taken to dryness, dissolved in 50 μL of 0.01 M HCl, and added to 20 mL of Aquasol (New England Nuclear Corp.). Scintillation counting was performed on a programmable LKB 1215 RackBeta scintillation counter equipped with automatic quench correction and a disintegrations per minute converter.

[1] Abbreviations used: Dnp-, 2,4-dinitrophenyl-; Dnp-F, 1-fluoro-2,4-dinitrobenzene. Myeloma proteins are designated by their parent plasmacytoma lines.

Table I: Summary of Reactivity Data for the Three Immunoglobulins[a]

residue		MOPC 167 IgA	MOPC 104E IgM	UPC 10 IgG2a
N-terminal Asp	pK	8.54 ± 0.03		8.10 ± 0.04
	r	39.1 ± 1.1		35.1 ± 0.7
	res/mol[b]	1(L chain)		1(L chain)
N-terminal Glu	pK	8.10 ± 0.03	8.02 ± 0.02	8.32 ± 0.08
	r	27.8 ± 0.6	25.2 ± 0.3	81.6 ± 4.0
	res/mol	1(H chain)	2(H and L chain)	1(H chain)
His	pK	7.25 ± 0.07	7.31 ± 0.12	7.34 ± 0.04
	r	7.90 ± 0.32	7.72 ± 0.44	2.12 ± 0.04
	res/mol	10.2	15.2	11.8
Cys	pK	9.22 ± 0.18	8.50 ± 0.10	
	r	175 ± 42	39.0 ± 3.1	
	res/mol	~1.3	~0.1	
Lys	pK	9.59 ± 0.11	9.71 ± 0.14	10.37 ± 0.02
	r	462 ± 84	222 ± 58	274 ± 6
	res/mol	34.2	46.5	42.7
Tyr	pK	9.76 ± 0.12	10.01 ± 0.39	10.01 ± 0.02
	r	299 ± 68	283 ± 222	240 ± 4
	res/mol	27.0	29.4	27.4

[a] The values of pK and reactivity and their standard errors were calculated by fitting the data to theoretical titration curves using a nonlinear least-squares procedure. [b] res/mol = residues per mole of H chain plus L chain. The possible contributions of J chain to the IgA and IgM N termini have been ignored. IgA and IgG compositions were obtained by amino acid analysis; the IgM composition was calculated from sequence data (Appella, 1971; Kehry et al., 1979) plus a weighted contribution from mouse J chain using the composition data of Barger & Inman (1976). For cysteine assays, see the text.

The second-order velocity constants for the reaction of Dnp-F with each class of functional group relative to the internal standard imidazolelactate were calculated by using the equation (Kaplan et al., 1971)

$$\alpha_x r = \alpha_s(^3H/^{14}C)_x/(^3H/^{14}C)_s$$

where α_x is the degree of ionization of the functional group under study, α_s is the degree of ionization of the imidazolelactate, pK = 7.27 (Cruickshank & Kaplan, 1975), r is the pH-independent second-order velocity constant for the reaction of the functional group under study relative to that for the internal standard imidazolelactate, and $(^3H/^{14}C)_x$ and $(^3H/^{14}C)_s$ are the corresponding ^3H to ^{14}C disintegrations per minute ratios for the group and internal standard.

Results and Discussion

Table I summarizes the pK and reactivity values determined for the various classes of functional group in the three immunoglobulins. Since alkaline denaturation is to be expected at high pH, the pK and r values (reactivity of the deprotonated group relative to the deprotonated imidazole of the internal standard) are estimated by extrapolation of data obtained below pH 9.5. As a consequence, the precision of values obtained for groups with high pK, lysine and tyrosine, is less than that for other groups. The majority of the data obtained fit theoretical titration curves well, with no obvious major discontinuities. Though deviations could have been expected since the overall ionization of the protein might affect the behavior of a functional group and there could be individual environmental differences between groups of a particular type, evidently these factors are of minor significance in most cases.

Apparently, for each class of functional group, there is a set that predominates in the data, and for a small number of residues to affect the appearance of the titration curves, their properties would have to be substantially different from those of the majority. This is probably the case in the histidine data for the IgG (see below).

All properties reported were determined simultaneously; the total amounts of protein used were 8 (IgG2a), 21 (IgA), and 38 mg (IgM).

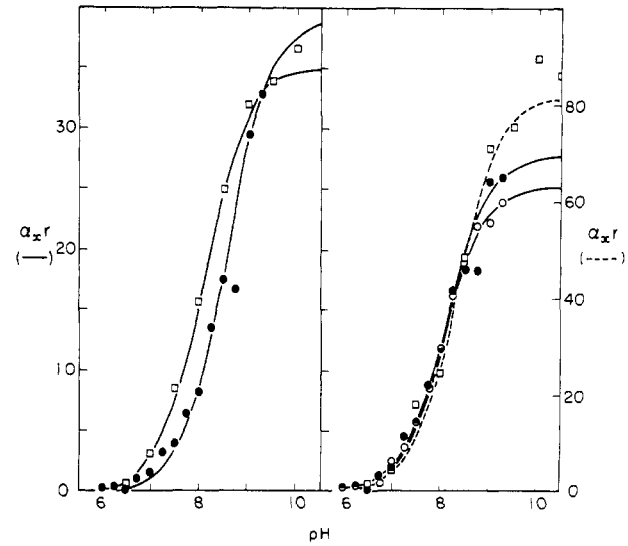

FIGURE 1: pH–reactivity profiles of the N-terminal residues of the heavy and light chains of the three immunoglobulins: MOPC 167 IgA (●), UPC 10 IgG2a (□), and MOPC 104E IgM (○). (Left panel) Aspartic acid N termini of the light chains; (right panel) glutamic acid N termini of the heavy chains; the IgM data are the average of the heavy and light chains since both bear glutamic acid N termini. The lines are theoretical titration curves whose parameters are given in Table I. Note the different scale used for the IgG2a data in the right panel.

N-Terminal Residues. Figure 1 presents the data obtained for the N-terminal residues, aspartic acid and glutamic acid, found in the three proteins, together with the fitted titration curves. X-ray crystallographic studies [e.g., Saul et al. (1978)] have shown that the N-terminal residues of immunoglobulins lie on the surface of the variable domains. The reactivities and pK values we have found conform to this view, though there is a significant difference in the pK of aspartic acid residues, ~0.4 unit, in the κ light chains of the IgG and the IgA, and the glutamic acid residue from the heavy chain of the IgG is distinguishable from its counterparts in IgA and IgM. The three proteins do not share any V-domain isotypes (Potter, 1977) so these variations in properties could be due

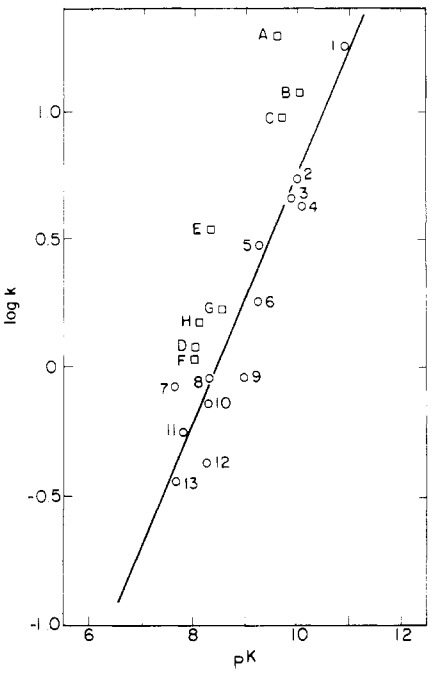

FIGURE 2: Brønsted plot of data (H. Kaplan, Y. K. Chan, and G. Oda, unpublished observations) for the reaction of a series of standard primary amines with Dnp-F (○), together with the data obtained for amino groups of the immunoglobulins (□). 1, α-Cbz-Lys; 2, Gly; 3, Leu; 4, Ala; 5, Phe; 6, Gln; 7, Gln-Gly; 8, $(Ala)_5$; 9, Asn; 10, Ala-Gly; 11, Phe-Gly; 12, $(Gly)_6$; 13, $(Phe\text{-}Gly)_2$; A, IgA Lys; B, IgG Lys; C, IgM Lys; D, IgA N-terminal Glu; E, IgG N-terminal Glu; F, IgM N-terminal Glu; G, IgA N-terminal Asp; H, IgG N-terminal Asp. The line is a least-squares fit of the standard data; $\log k = (0.455 \pm 0.045)pK - (3.86 \pm 0.40)$.

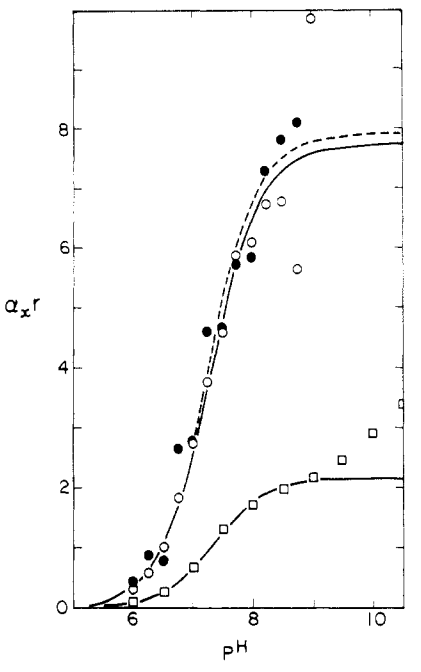

FIGURE 3: pH–reactivity profiles of the histidine residues of the IgA (●), IgG2a (□), and IgM (○). The lines are the theoretical titration curves whose parameters are given in Table I.

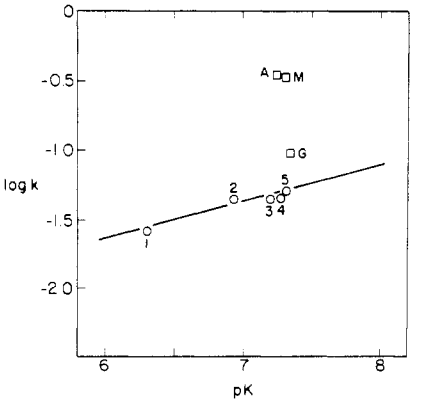

FIGURE 4: Brønsted plot of data (Cruickshank & Kaplan, 1975) for the reaction of a series of standard imidazole compounds with Dnp-F (○), together with the data obtained for the histidines of the IgA (A), IgG2a (G), and IgM (M). 1, 4-(hydroxymethyl)imidazole; 2, imidazole; 3, α-*N*-acetyl-L-histidine; 4, β-L-imidazolelactic acid; 5, imidazolylacetic acid.

to sequence differences in the first framework segment.

In the case of the two aspartic acid N-terminal residues and the IgG glutamic acid, the residues are unique. For the IgA and the IgM, the data for the N-terminal residues may contain some contribution from the J chain. Mouse J chain may well be homologous to human J chain whose N-terminal residue is pyrrolidonecarboxylic acid (Mole et al., 1977), a proportion of which might be in the open glutamic acid form. For the IgM, both heavy and light chains bear glutamic acid N-terminal residues and the data should be the average of the two. The data indicate that the N-terminal residues of the L and H chains are very similar in character in the IgM. The yields we obtained of the radiolabeled Dnp-Glu suggested that very little cyclization to pyrrolidonecarboxylic acid had occurred in our samples, though a low yield in Edman degradation of the H chain of MOPC 104E IgM has been reported (Robinson et al., 1973). Possibly the additional manipulations needed to separate the chains for sequence work were responsible for the cyclization encountered. The excellent fit of the data to theoretical titration curves indicates that the variable domains did not undergo any major conformational change through the pH region examined.

By multiplying the r values by the known second-order velocity constant, k, for the reaction of the internal standard imidazolelactate with Dnp-F, 0.0443 M^{-1} min^{-1} (Cruickshank & Kaplan, 1975), k values for the N-terminal amino groups can be obtained. These values are compared in a Brønsted plot (Figure 2) with values for model compounds. Only the glutamic acid N-terminal residue of the IgG deviates significantly; its reactivity was higher than that of a standard amine of the same pK. The increased reactivity indicates some interaction of the N-terminal residue with neighboring elements of the protein structure. The properties of N-terminal amino groups have led to their utilization in functional roles in proteins such as chymotrypsin (Mathews et al., 1967) and hemoglobin (Arnone, 1974). Such groups deviate much more from the Brønsted plot; hence, it may be inferred that N-terminal amino groups in immunoglobulin probably play no functional role, only a passive structural one.

Histidine Residues. The ionization range of imidazole side chains is expected to be near neutral pH. The data in Figure 3 show that histidines in immunoglobulins are normal in this regard, but their reactivities are substantially greater than expected. This is apparent from the $\alpha_x r$ values in Figure 3 which express the reactivities relative to the free imidazole of the internal standard imidazolelactate. The Brønsted plot in Figure 4 shows that these groups are indeed more reactive than expected, from comparison with a series of standard imidazole compounds (Cruickshank & Kaplan, 1975).

The properties of the histidines of the IgA and the IgM are remarkably similar, whereas IgG has histidine groups of similar pK but much lower reactivity. The excellent fit of the data

to theoretical titration curves indicates that there are not blocks of histidine residues of substantially different character in the proteins. Up to pH 8.5 there are no indications of changes in the microenvironment of the histidine groups, but above this pH the data showed major deviations from the titration curves and in the case of IgG a different process appears to be occurring, giving slightly enhanced reactivity. This may reflect the second ionization of the imidazole moiety.

It has previously been observed that protein environments can cause significant enhancements of the reactivities of imidazole moieties: e.g., in lysozyme (Duggleby & Kaplan, 1975), in chymotrypsin (Cruickshank & Kaplan, 1975), and in insulin (H. Kaplan, Y. K. Chan, and G. Oda, unpublished observations). This has been attributed to hydrogen bonding of the imidazole moiety to other groups in the protein. It would therefore appear that in the IgA and the IgM the majority of the histidine groups are hydrogen-bonded, while in IgG a substantial number of the histidines are in a quite different microenvironment. Two features in the distribution of histidine residues in the amino acid sequences are noteworthy. First, in immunoglobulin domains, a histidine residue frequently occurs three or four residues after the cysteine that is the C-terminal component of the domain's internal disulfide bridge (Beale & Feinstein, 1976). In the four constant domains of the IgG2a, 3 of the 10 histidines are of this type, and in the five constant domains of the IgM, there are five such histidines; there are 12 histidines in constant domains of the IgM's total of 15 per H and L chain. Second, several histidines are relatively close, considering the overall size of the proteins. In the IgM there are triplets of histidines at residues 191, 200, and 203 in the L chain sequence (Appella, 1971) and at residues 430, 448, and 450 in the H chain (Kehry et al., 1979), and in the IgG2a a triplet of histidines occurs at residues 285, 291, and 310 and at residues 429, 433, and 435 in the heavy chain (Bourgois et al., 1974).

Cysteine Residues. Immunoglobulins do not bear free thiol groups often. However, in the case of mouse IgA, thiol groups have been reported in the Fd region of the heavy chain (Rosenstein et al., 1972; Jackson & Richards, 1974). Also, its light chains are not attached by disulfide bridges to the heavy chains and either can be monomeric, and so potentially bear a thiol group, or form a disulfide-bonded dimer (Abel & Grey, 1968). Thiol analyses of several MOPC 167 IgA preparations (S. A. Cockle and N. M. Young, unpublished results) indicate five to six thiol groups per mole of IgA dimer. In view of the many disulfide bridges present in IgA molecules (Liu et al., 1976), it is possible that some thiol may arise from incomplete disulfide bridge formation during biosynthesis. This may also be the explanation for the trace amount of thiol group we found in our IgM preparations (0.54–0.95 mol/mol of IgM) and also reported by Miller & Metzger (1965) for a human IgM. Other possibilities are a contribution from J chain or insertion of thiol-bearing proteins such as albumin into disulfide bonds of the immunoglobulins, resulting in new thiol groups on the immunoglobulins.

The behavior of the IgA and IgM thiol groups is shown in Figure 5. In comparison to the N-terminal and histidine residues, the fit of the data to theoretical titration curves is relatively poor, particularly in the lower end of the pH range. This suggests some heterogeneity of the thiol groups being examined. The pK values obtained are 0.7 unit apart with the IgA one being closer to the expected value. The IgM thiol group also has substantially lower reactivity than that of the IgA (Table I). Because of the high nucleophilicity of deprotonated thiol groups, it is expected that their reactivities

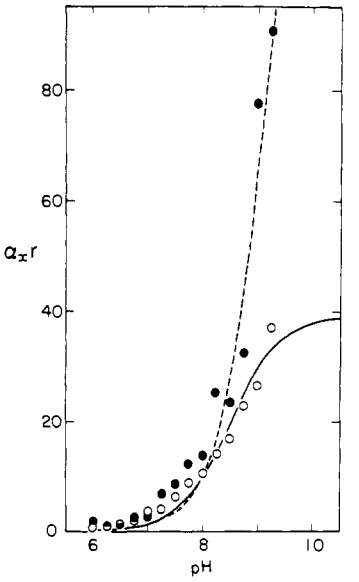

FIGURE 5: pH–reactivity profiles of the cysteine residues of the IgA (●) and IgM (○). The lines are the theoretical titration curves whose parameters are given in Table I.

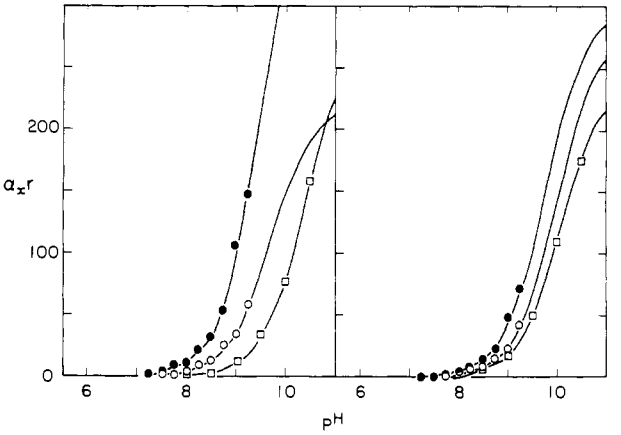

FIGURE 6: pH–reactivity profiles of the lysine (left panel) and tyrosine (right panel) residues of the IgA (●), IgG2a (□), and IgM (○). The lines are the theoretical titration curves whose parameters are given in Table I.

should be greater than the reactivities of the α-amino groups of the N-terminal residues, but this is not found for the IgM thiol group. Lacking the data for standard thiols needed to construct a Brønsted plot, it is not possible to evaluate the reactivities in a more quantitative manner.

Lysine and Tyrosine Residues. As pointed out above, the high pK of these groups makes determination of their properties less reliable. In spite of this, only the tyrosine data for the IgM show major uncertainties in the parameters (Table I). The data and titration curves for the lysine and tyrosine residues are shown in Figure 6. It appears that the tyrosine residues of the IgA are of significantly lower average pK than the other two immunoglobulins, but the reactivities are all similar. A wider spread of behavior is evident in the lysine data with the IgA lysines being distinct from the IgG lysines in both pK and reactivity while the IgM lysines have intermediate properties, resembling the IgA residues in pK and the IgG residues in reactivity. This spread of behavior is also seen in the Brønsted plot (Figure 2). The apparently normal behavior of the IgM lysines suggests that salt linkages involving lysine residues are not significant factors in stabilizing the pentameric arrangement of the Fc subunits of the IgM.

The reactivity of *N*-acetyltyrosinamide with Dnp-F has been determined (H. Kaplan, Y. K. Chan, and G. Oda, unpublished observations), and its second-order velocity constant is 29 M^{-1} min^{-1}. The *r* values obtained for the tyrosine residues (Table I) are equivalent to constants of 10.64 (IgG), 12.54 (IgM), and 13.25 (IgA) M^{-1} min^{-1}. These values are significantly lower than that of the standard compound, pointing to several tyrosine residues being involved in specific interactions with other elements of the protein structures. For comparison, the average velocity constant of the four tyrosines in insulin was 2.2 M^{-1} min^{-1} (Y. K. Chan, G. Oda, and H. Kaplan, unpublished observations).

The sequence data show lysines to be distributed throughout the molecules, with few in homology positions of the type noted above for histidine residues. A difference between the IgM and the IgA is the much larger content of lysine in the MOPC 104E V_H domain, 10 residues compared to 4 in MOPC 167, only 2 being homologous. There are also eight lysines in the unique C_H2 domain of the IgM. On the other hand, the majority of the tyrosine residues of the V_H and V_L domains of MOPC 167 and MOPC 104E, seven out of eight and nine tyrosines, respectively, are in homologous positions, and several homologies occur in the constant domains. The p*K* and reactivity data (Table I) are in accord with these sequence data since there is a greater spread in the average behavior among the lysines than among the tyrosines of the three proteins.

Conclusion

In the experiments we have described, all the classes of functional group in the immunoglobulins showed substantial reactivities. With the possible exceptions of the IgA and IgM histidines, no functional groups behaved in a fashion that might have indicated some unusual structure in the proteins. Since the data could be fitted by simple titration curves, the properties of the various groups remained constant and hence the domains of the immunoglobulins appear stable in the pH range employed. These factors, together with the modest amounts of protein required for the determination of the properties, indicated that the study of changes in the group properties, that may be produced by interaction with antigen or proteins of effector systems such as Clq, should be feasible by the competitive labeling approach. By using the many specific cleavages known for immunoglobulins, it should be possible to examine individual residues in the various domains and relate their chemical properties to biological effects.

After this manuscript was submitted, an NMR study of histidines in the pFc′ fragment of rabbit IgG was published (Boyd et al., 1979). This study showed that IgG has histidines of abnormally high p*K*, as indicated by the data in Figure 3.

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Obligatory Hybridization of Heterologous Immunoglobulin Light Chains into Covalently Linked Dimers†

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ABSTRACT: Immunoglobulin light chains, which generally show preference for homologous association in forming dimers, can be forced to hybridize with heterologous molecules in yields of 80–100%. Light chains (λ type) from the patient Mcg were S-sulfonated at the penultimate half-cystine residues of the interchain disulfide bond. The interchain half-cystine residues of λ- or κ-type light chains from a second source were reduced, and the two samples were mixed. A hybrid was produced with formation of an interchain disulfide bond between heterologous light chains. The Mcg light chain (Bence-Jones) dimer crystallizes readily, and several hybrids containing the Mcg light chain also crystallized. The affinity of the Mcg dimer for bis(dinitrophenyl)lysine was also passed on to the hybrids. For example, the Mcg × Weir hybrid showed binding affinity for the ligand, while the parent Weir dimer did not. The Mcg and Vil λ chains are identical in amino acid sequence in their first hypervariable regions and are similar throughout the variable domains. Both the Vil dimer and the Mcg × Vil hybrid bound bis(dinitrophenyl)lysine. The Tew κ chain, like the Mcg protein, was obtained from a patient with amyloidosis and included in its sequence all but one of the side chains associated with dinitrophenyl ligand binding in the Mcg dimer. The Tew dimer and the Mcg × Tew hybrid showed the expected binding of bis(dinitrophenyl)lysine.

The initial impetus for this work came from Dr. Elvin A. Kabat in 1977. He suggested that crystal-structure analyses of hybrids of the Mcg light chain and such closely related proteins as the Vil λ chain would contribute to the understanding of the specificity and geometry of complementarity-determining regions in immunoglobulins (Kabat et al., 1977; Fett & Deutsch, 1974, 1976; Ponstingl & Hilschmann, 1969).

The use of the Mcg protein for hybridization is advantageous because the three-dimensional structure has been determined and correlated with the amino acid sequence (Schiffer et al., 1973; Edmundson et al., 1975; Fett & Deutsch, 1974, 1976). Moreover, the binding of hapten-like molecules to the Mcg Bence-Jones dimer has been studied both in crystals and in solution (Edmundson et al., 1974; Firca et al., 1978).

The principal obstacle to success was the preference of light chains for self- (homologous) association rather than heterologous dimerization (Stevenson & Straus, 1968). In the absence of an intact interchain disulfide bond, light chains participate in monomer–dimer equilibria (Stevenson & Dorrington, 1970; Björk & Tanford, 1971). The dimer is stabilized by noncovalent interactions between pairs of like domains (i.e., V_l–V_2 and C_l–C_2 pairs[1]). Assuming that heterologous light chains form noncovalent dimers, even in minute amounts, we searched for a method to prevent dissociation by locking the components together with an interchain disulfide bond. In the present article we describe such a method and its application to both λ and κ chains.

Materials and Methods

Preparation of Bence-Jones Proteins. Patients with multiple myeloma and/or amyloidosis were screened for the presence of urinary Bence-Jones proteins. Urine was collected from patients in which the quantities of Bence-Jones protein exceeded about 1 g/L. The protein was precipitated by the addition of ammonium sulfate to 90% saturation. After centrifugation, the pellet was washed twice with 75% saturated ammonium sulfate and stored as an ammonium sulfate paste at −10 °C.

Samples of proteins or urines were kindly supplied by Drs. H. F. Deutsch of the University of Wisconsin (Mcg and Weir), N. Hilschmann of the Max-Planck Institute in Göttingen, West Germany (Vil), A. Solomon of the University of Tennessee (Hud and May), E. F. Osserman of Columbia University (Cot, Ste, Joy, and Tew), and T. M. Cosgriff (All) and B. D. Cheson of the University of Utah (Bla). The Tew protein was a κ chain and all other proteins were λ chains. Of the proteins for which partial or complete amino acid sequences are known, the Vil and Weir λ chains have been assigned to subclass II, the Mcg protein to subclass V, and the Tew κ chain to subclass II (Ponstingl & Hilschmann, 1969; Fett &

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[1] Abbreviations used: V, variable domain; C, constant domain; DEAE, diethylaminoethyl; MEA, 2-mercaptoethylamine; MAA, 2-mercaptoacetic acid; Nbs_2, 5,5′-dithiobis(2-nitrobenzoate); Nbs, 5-thio-2-nitrobenzoate; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; EDTA, ethylenedinitrilotetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Dnp, 2,4-dinitrophenyl.

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