Proton Nuclear Magnetic Resonance Studies of Human Immunoglobulins. Solution Conformation of the Constant Domain of the λ Light Chains and Identification of the Isotypes†

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ABSTRACT: Sixteen λ-type Bence-Jones proteins and eight constant domain fragments of λ chains were used to measure nuclear magnetic resonance (NMR) signals of the C2- and C4-H protons of two histidine residues (His-189 and His-198) which exist in the constant half of the λ chains. The pH titration curves for the C2-H proton signals of His-189 and His-198 of six Bence-Jones dimers were compared with those for the corresponding constant fragments. It was concluded that the tertiary structure of the constant domain is well preserved even in the constant fragments which are known to exist as the monomer in solution. It was shown that the C2- and C4-H proton peaks of His-198 can be used to identify the Mcg *isotype*. It was also shown that the chemical shifts of the C4-H proton of His-189 and His-198 can be used to detect the amino acid substitutions at positions 153 and 190 which are the Kern and Oz markers, respectively. On the basis of the NMR measurements, it was suggested that the Kern and at least one of the Mcg markers are in close spatial proximity to His-189 and His-198, respectively. The NMR data were compared with the X-ray structure of the Fab fragment of human myeloma immunoglobulin, IgG1 (λ) New [Poljak, R. J., Amzel, L. M., Avey, H. P., Chen, B. L., Phizacherley, R. P., & Saul, F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3305–3310]. The NMR data are explained in terms of the crystal structure and are thus consistent with solution and crystal structure being quite similar in the constant domain of the λ chain.

Light chains of immunoglobulins are divided into two homology units (domains) of about 110 amino acid residues. The variable, amino-terminal domain differs markedly from one light chain to another, whereas the constant, carboxy-terminal domain has essentially an invariant sequence. The light chains exist in two types, λ and κ, the structural differences of which are reflected in antigenic differences. Bence-Jones proteins, which are excreted into the urine of patients with mulitple myeloma, are dimers of homogeneous light chains. In the constant domain of λ chains, alternatives have been found at several positions, including positions 113 (Ala-Asn), 115 (Ser-Thr), 153 (Ser-Gly), 164 (Thr-Lys), and 190 (Arg-Lys).[1] Oz(+) λ chains have lysine at 190, Kern(+) λ chains have glycine at 153, and Mcg(+) λ chains have asparagine at 113, threonine at 115, and lysine at 164. These variants are known to be *isotypes*, which exist, along with Oz(−), Kern(−), and Mcg(−) λ chains, in all normal individuals (Ein & Fahey, 1967; Ponstingl et al., 1968; Fett & Deutsch, 1974; Solomon, 1977). It is known that Oz, Kern, and Mcg isotypes can be identified by a serological method (Ein & Fahey, 1967; Ponstingl et al., 1968; Solomon, 1977).

It should be emphasized that the serological method does not always give an unambiguous result for the detection of heterogeneities in immunoglobulin molecules (Tsuzukida et al., 1979). In general, it would be quite useful to have a simple and reliable alternative means available to detect various types of molecular heterogeneities in the immunoglobulin molecules.

In a previous paper, we have reported assignments of nuclear magnetic resonance (NMR)[2] peaks of the C2-H proton of two histidine residues (His-189 and His-198) which are present in common in the constant domain of λ chains (Arata et al., 1978a). On the basis of the NMR data on the pK_a values, the rates of proton–deuterium exchange at the C2 position, and the line widths for His-189 and His-198, it was concluded that these two histidine residues are located in quite different environments; His-189 is exposed to solvent, whereas His-198 is buried and not accessible to solvent (Arata & Shimizu, 1979). The C2-H protons of His-198 of 15 λ chains examined gave two distinct types of pH titration curves, and we suggested that these two types of titration curves correspond to Mcg(+) and Mcg(−) λ chains (Arata & Shimizu, 1979). In the present work, the isotypes of six λ chains were determined by the amino acid composition of tryptic peptides purified by conventional methods. Constant domain fragments (C_L) were obtained by limited papain digestion. The results of NMR measurements of eight C_L fragments with the known isotypes are used along with those of a number of λ chains previously reported by us (Arata & Shimizu, 1979) to discuss the conformation of the constant domain in the intact λ chains, as well as in the C_L fragments, in solution. We will show that C2- and C4-H proton chemical shifts of the two constant domain histidines can actually be used to identify the Mcg isotype. It will also be shown that the Kern and Oz markers at positions 153 and 190, respectively, can be identified by using the C4-H proton signals of His-189 and His-198. The results obtained for the intact λ chains and the C_L fragments will be discussed in terms of X-ray structural studies of the constant domain of λ chains (Poljak et al., 1973; Edmundson et al., 1975).

Materials and Methods

Preparation of λ Chains and Their C_L Fragments. Bence-Jones proteins Ak, Hiro, Jon, Mae, and Uts[3] were

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[1] The numbering system used in the present paper is based on the protein Sh; see Putnam et al. (1967).

[2] Abbreviations used: C_L, the constant fragment of the light chain; DSS, sodium 4,4-dimethyl-4-silapentanesulfonate; $NaDodSO_4$, sodium dodecyl sulfate; NMR, nuclear magnetic resonance; Tos-PheCH_2Cl, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.

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collected from the urine of patients with multiple myeloma. The proteins were precipitated with ammonium sulfate and purified by column chromatography on DEAE-cellulose (DE-52, Whatman) (Bernier & Putnam, 1964). The purity and the ratio of the disulfide-linked dimer and the other forms of the protein in each preparation were checked by NaDodSO$_4$–polyacrylamide gel electrophoresis and also by gel filtration on Sephadex G-100 in 7% propionic acid. λ-Type Bence-Jones proteins Blo, Kern, Kob, Mcg, NIG58, Sh, Vi, and Weir were provided by other laboratories.[3] The C_L fragment was prepared by papain digestion according to the method of Karlsson et al. (1969) with minor modifications. The purified λ chain was dissolved in 0.2 M Tris-HCl, pH 8.5 (1%), reduced by adding dithiothreitol (10 mM), and alkylated with iodoacetamide (22 mM). The reduced, alkylated protein was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) containing 1.0 mM cysteine and 0.2 mM EDTA, and digested with mercuripapain (Sigma) at 37° C for 10 min to 2 h. In order to achieve a maximum yield, it was necessary to adjust the reaction time and the papain/λ chain ratio, depending upon the chain to be cleaved. For example, 200 mg of reduced, alkylated λ chain Uts was dissolved in 20 mL of the phosphate buffer and digested with 0.4 mg of papain for 30 min at 37 °C. Iodoacetamide was added at a final concentration of 5 mM to terminate the reaction. The digests were subjected to gel filtration at 5 °C on a Sephadex G-100 column (5 × 100 cm) equilibrated with 0.1 M Tris-HCl, 0.15 M NaCl, and 0.01% NaN_3 at pH 8.0. The fraction for the C_L fragment was collected and was further purified on a DEAE-cellulose column (DE-52, Whatman, 2.5 × 15 cm) equilibrated with 0.01 M Tris-HCl, pH 8.0. The yield of the C_L fragment Uts was 70 mg. The C_L fragments of proteins Ak, Hiro, Jon, Kern, Kob, and Mae were obtained by a similar procedure. Two kinds of C_L fragments of protein Nag, which had been obtained by papain and trypsin digestions, were kindly provided by Dr. K. Hamaguchi. It was confirmed that each one of the C_L fragments used in the present experiments gives a single band on cellulose acetate electrophoresis and reacts with anti-λ chain sera, giving a single arc. The amino acid composition of the C_L fragments is in good agreement with that predicted from the known sequence of the λ chains. The molecular weight of each C_L fragment as determined by gel filtration is about one-fourth of the corresponding intact Bence-Jones dimer.

A peptide with the amino acid composition $Gly^{-}_{1.00}Glu_{1.08}Pro_{0.89}Lys_{0.97}$ was recovered from the tryptic digests of the C_L fragment Uts in good yield (40%). The same peptide was obtained from the C_L fragments Ak, Hiro, and Jon. The N-terminal sequence of the C_L fragment Uts was determined as Gly^{108}-Gln-Pro-Lys-Ala- by the dansyl-Edman method (Gray, 1972). The N-terminal residue of the C_L fragments Ak, Hiro, Jon, Kob, and Mae was determined as glycine; the C_L fragment Kern was shown to have serine at its N terminus. The N-terminal sequence of the C_L fragment Nag obtained by papain digestion is Ser^{108}-Gln-Pro- (Goto & Hamaguchi, 1979); the C_L fragment Nag obtained by trypsin digestion has the N-terminal sequence Leu^{104}-Thr-Val-Leu-Ser-Gln-Pro- (Goto et al., 1979).

Isolation of Key Peptides. Forty milligrams of each C_L fragment was dissolved in 10 mL of deionized water and digested with 0.8 mg of trypsin (Tos-PheCH_2Cl-trypsin, Sigma) at 37 °C for 2 h. During the digestion the pH was maintained between 8.0 and 8.5 with 0.1 M NaOH. The tryptic digests dissolved in 2 mL of ammonium bicarbonate buffer (0.01 M, pH 9.0) were applied to a DEAE-Sephadex A-25 column (0.9 × 50 cm) and eluted at 20 °C with ammonium bicarbonate buffer of increasing molarity (0.01–0.5 M), pH 9.0. The eluate was monitored by the optical density at 230 nm. Relevant peptide fractions were pooled and lyophilized, and the purity of each peptide was checked by paper chromatography (the organic phase of butanol–acetic acid–water (4:1:5)) and by high-voltage paper electrophoresis (pH 3.7). Isolation, amino acid analysis, and the determination of the partial sequence of the peptides were carried out by conventional methods.

NMR Measurements. ^{1}H NMR spectra were recorded on a JEOL PS-100 spectrometer operating at 100 MHz in the correlation mode (Arata & Ozawa, 1976; Arata et al., 1978b). Ten milligrams of each light chain or 5 mg of each C_L fragment was dissolved in 0.3 mL of 0.2 M NaCl–D_2O. The pH was adjusted with 0.3–0.5 M DCl or NaOD. All pH values reported in this paper are uncorrected meter readings of D_2O solutions made with an electrode standardized by using H_2O buffers. Typically, 2000 transients (512 Hz/0.5 s) were accumulated to improve the signal-to-noise ratio. All chemical shifts are given in parts per million from external DSS (5% in D_2O). The probe temperature was 29 °C throughout the experiment.

Results and Discussion

Chemical Determination of Isotypes. Figure 1 shows the elution profile on a DEAE-Sephadex A-25 column for the tryptic digests from the C_L fragments Ak and Uts. Peptides 1–6, which can be used to identify the Oz, Kern, and Mcg markers and also the N-terminal sequence of the C_L fragments, were eluted at an earlier stage and gave well-separated peaks. The amino acid compositions of peptides 1–6 are shown in Table I. Similar experiments were performed for the proteins Hiro, Jon, Kob, and Nag. These results clearly show that proteins Kob and Uts have sequences with Mcg(+), Kern(+), and Oz(−) markers, whereas proteins Ak, Hiro, and Jon are of Mcg(−), Kern(−), and Oz(−) isotypes; protein Nag has a sequence with the Mcg(−), Kern(−), and Oz(+) markers. These results, along with those reported from other laboratories, are summarized in Table II.

Isotype Identification by ^{1}H NMR. Figure 2 gives the pH dependence of the chemical shifts of the C2– and C4–H proton signals of His-189 and His-198 of eight C_L fragments. The C2–H proton of His-189 for all the C_L fragments gives titration curves which are in close agreement with each other. On the other hand, in the case of His-198, the C2– and C4–H titration curves can clearly be divided into two distinct types, A and B. The titration curve of type A with a pK_a of 5.0 is observed for proteins Kob and Uts, whereas that of type B with a pK_a of 4.5 is observed for proteins Ak, Hiro, Jon, Kern, and Mae. As summarized in Table I, proteins Kob and Uts are Mcg(+) and the other proteins are Mcg(−). In our previous paper, we have examined the C2–H proton titration curves for His-189 and His-198 of 15 intact λ chains and reported that the His-189 titration curves are homogeneous for all proteins examined, whereas His-198 titration curves show a significant degree of deviation for proteins Kob, Mcg, Uts and Weir (Arata & Shimizu, 1979). Proteins Mcg and Weir have been identified as Mcg(+), Kern(+), and Oz(−) isotypes with an additional substitution at position 157 in the case of the protein Weir (Fett & Deutsch, 1976). We have suggested that the large deviation observed for the His-198 titration is due

[3] The authors are indebted to the following people for samples of Bence-Jones proteins: Dr. T. Azuma and Dr. K. Hamaguchi, Osaka University; Dr. H. F. Deutsch, University of Wisconsin; Dr. N. Hilschmann, Max-Planck-Institute; Dr. T. Kitani and Dr. T. Yonezawa, Osaka Univeristy; Dr. S. Migita, Kanazawa University; Dr. F. W. Putnam, Indiana University; Dr. T. Shinoda, Tokyo Metropolitan University.

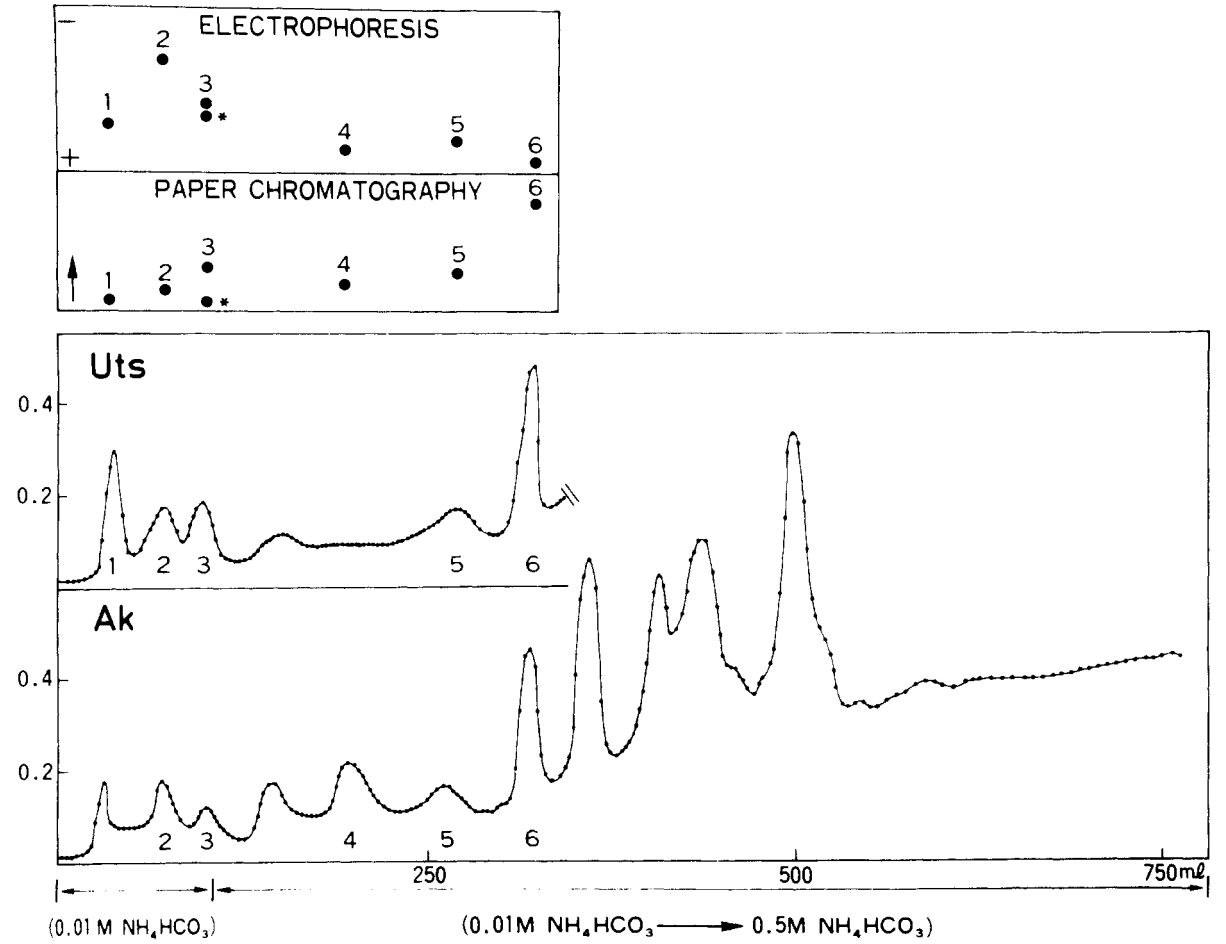

FIGURE 1: Separation of tryptic peptides of C_L fragments Uts and Ak (40 mg each) on a DEAE-Sephadex A-25 column (0.9 × 60 cm). Elution was performed with 0.01 M NH_4HCO_3, pH 9.0, and the ionic strength of the buffer was increased by a gradient change from 0.01 to 0.5 M NH_4HCO_3. The electrophoretogram and paper chromatogram for peptides 1–6 are also shown. Peak 3 in the column chromatogram actually consists of two peptides, i.e., Gly^{108}-Lys^{111} (3) and Gln^{168}-Lys^{172} (*). The amino acid compositions of peptides 1–6 are given in Table I.

Table I: Amino Acid Compositions of Peptides 1-6[a] Obtained by Tryptic Digestion of C_L Fragments Ak and Uts[b]

	Gly^{108}-Lys^{111}		Ala^{112}-Lys^{130}		Ala^{151}-Lys^{157}		Ala^{158}-Lys^{167}		Ser^{188}-Arg^{190}	
	Ak	Uts	Ak	Uts	Ak	Uts	Ak	Uts	Ak	Uts
Lys	0.96 (1)	0.97 (1)	0.99 (1)	1.05 (1)	0.89 (1)	0.89 (1)	0.96 (1)	2.19 (2)		
His									1.02 (1)	1.07 (1)
Arg									1.00 (1)	1.00 (1)
Asp			1.05 (1)	1.89 (2)	1.01 (1)	1.07 (1)				
Thr			1.00 (1)	1.75 (2)			2.72 (3)	1.86 (2)		
Ser			2.69 (3)	1.91 (2)	1.86 (2)	1.05 (1)	0.95 (1)	0.88 (1)	1.09 (1)	0.92 (1)
Glu	1.15 (1)	1.08 (1)	3.27 (3)	3.14 (3)			1.07 (1)	1.00 (1)		
Pro	1.03 (1)	0.89 (1)	3.15 (3)	3.19 (3)	1.07 (1)	1.12 (1)	0.96 (1)	0.81 (1)		
Gly	1.00 (1)	1.00 (1)				0.95 (1)	0.99 (1)	1.01 (1)		
Ala			3.00 (3)	2.00 (2)	1.00 (1)	1.00 (1)	1.00 (1)	0.86 (1)		
Val			1.07 (1)	1.03 (1)	0.93 (1)	0.93 (1)	0.99 (1)	0.80 (1)		
Leu			2.00 (2)	1.94 (2)						
Phe			1.07 (1)	0.67 (1)						
peak no.[a]	3	3	6	6	5	5	4	1	2	2
yield (%)	34	40	40	60	63	29	74	51	46	34

[a] See Figure 1. [b] Values given (residues/mole of peptide) are for 24-h acid hydrolysis. Expected values are in parentheses.

to the existence of Mcg(+) substitutions and that measurements of chemical shifts of the His-198 C2–H proton can be a simple means of identifying the Mcg isotype (Arata & Shimizu, 1979). In the present study it was confirmed that, as predicted by NMR, proteins Kob and Uts actually have Mcg(+) markers and that proteins Ak, Hiro, Jon, and Mae have Mcg(−) sequences. It should also be noted that protein Kern (Oz(−), Kern(+), Mcg(−)) gives type B titration curves for His-198 C2- and C4–H protons. We confirmed that intact λ chains also give two types of titration curves for the His-198 C4–H proton, as observed for the C_L fragments. In view of the results described so far, we conclude that the heterogeneity

Table II: Types of His-189 and His-198 NMR Titration Curves and Isotypes of 14 λ Light Chains

λ chains	NMR titration curves: intact λ chains, His-198 C2-H	C_L fragments: His-198 C2-H	C_L fragments: His-198 C4-H	C_L fragments: His-189 C4-H	isotypes: Oz	Kern	Mcg	ref
Mcg	A				−	+	+	Fett & Deutsch (1974)
Weir	A				−	+	+	Fett & Deutsch (1976)
Uts	A	A	A	C	−	+	+	present work
Kob	A	A	A	C	−	+	+	present work
Sh	B				−	−	−	Wikler et al. (1967)
Kern		B	B	C	−	+	−	Ponstingl et al. (1968)
Blo	B				+	−	−	Lieu et al. (1977)
Vi	B				−	−	−	Fett & Deutsch (1975)
Ak	B	B	B	D	−	−	−	present work
Hiro	B	B	B	D	−	−	−	present work
Jon	B	B	B	D	−	−	−	present work
Mae	B	B	B	D	−	−	−	Takahashi et al. (1979)
Nag		B	*a*	*a*	+	−	−	present work
NIG58	B				−	−	−	Takayasu et al. (1979)

[a] See text.

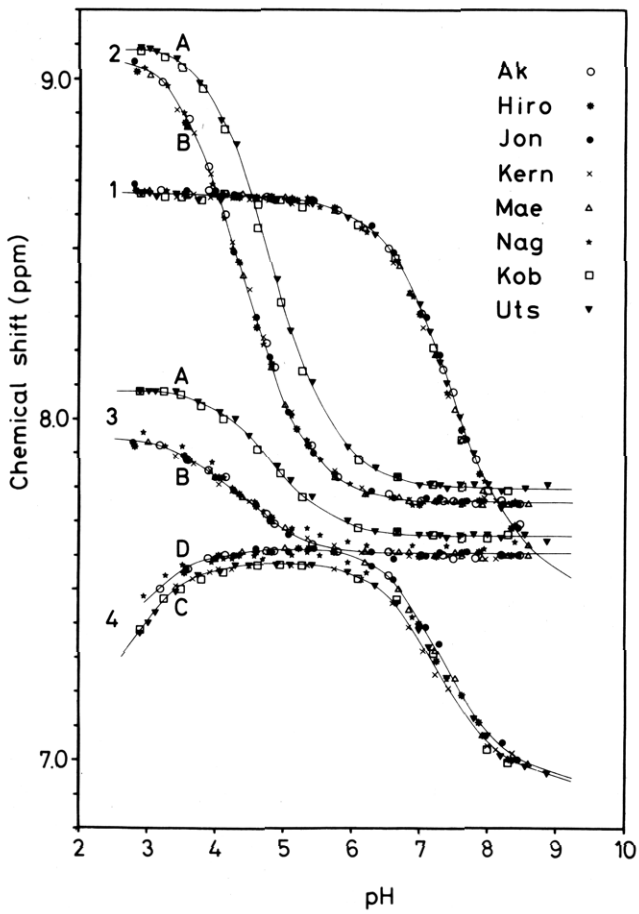

FIGURE 2: The pH dependence of the chemical shifts of the C2- and C4-H proton peaks of His-189 and His-198 of eight C_L fragments. Each C_L fragment was dissolved in 0.2 M NaCl-D_2O, 29 °C. Chemical shifts are in parts per million from external DSS (5% in D_2O). Assignments: (1) C2-H (His-189); (2) C2-H (His-198); (3) C4-H (His-198); (4) C4-H (His-189).

observed in the His-198 titration curves actually reflects the existence of the Mcg markers. Figure 3 shows the range for the His-189 and His-198 C2-H proton titration curves drawn using our previous data on intact λ chains; the shaded area for the titration curves corresponds to the scatter observed for 15 Bence-Jones proteins. With chemical-shift data between pH 4 and 5 of the His-198 C2-H proton of a given λ chain, Figure 3 can be used to identify the Mcg isotype.

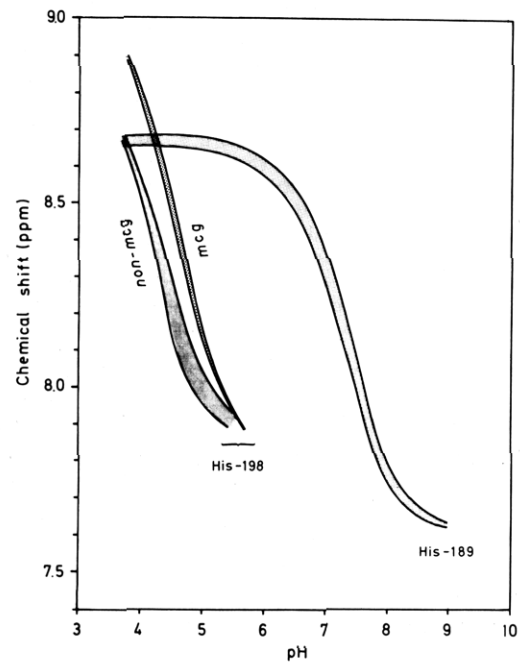

FIGURE 3: The pH-titration curves for the C2-H proton of the constant domain histidines of intact λ chains with Mcg and non-Mcg isotypes. Drawn using the data reported in our previous paper (Arata & Shimizu, 1979); all measurements were made in 0.2 M NaCl-D_2O, 29 °C, and the chemical shifts are in parts per million from external DSS (5% in D_2O).

It should also be noted in Figure 2 that the His-189 C4-H proton chemical shifts show a small but significant degree of heterogeneity. It will be described later that the heterogeneity of the His-189 C4-H proton chemical shifts can be more clearly observed for intact λ chains. Protein Kern, as well as proteins Kob and Uts, give quite similar titration curves (type C). By contrast, proteins Ak, Hiro, Jon, and Mae give titration curves of the type D, which are shifted to the low-field side and become inseparable from those of His-198 C4-H proton in the pH range 5–6. Proteins Kob, Uts, and Kern give separate His-189 and His-198 C4-H proton signals in the entire pH range. These differences are particularly distinct in the pH range 5–6, where the chemical shifts of the His-189 and His-198 signals change with pH only to a small extent. The differences may more clearly be seen in Figure 4, which gives the histidine C2- and C4-H proton spectra observed at pH

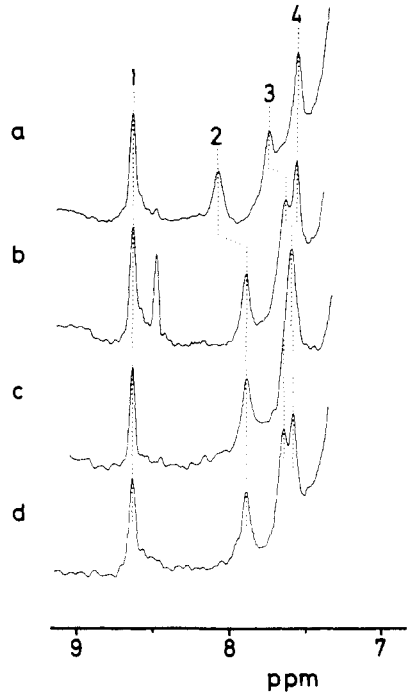

FIGURE 4: The 100-MHz ^{1}H NMR spectra of His-189 and His-198 of C_L fragments: (a) Uts (Oz(−), Kern(+), Mcg(+)) (5.48); (b) Kern (Oz(−), Kern(+), Mcg(−)) (5.44); (c) Mae (Oz(−), Kern(−), Mcg(−)) (5.41); (d) Nag (Oz(+), Kern(−), Mcg(−)) (5.45). The numbers in parentheses are the pH values at which the NMR spectra were observed. Assignments: (1) C2–H (His-189); (2) C2–H (His-198); (3) C4–H (His-198); (4) C4–H (His-189). Signals observed at 8.46 ppm, most clearly in the case of protein Kern, are probably due to an unfolded form of the protein.

5.4–5.5 of C_L fragments Uts, Kern, Mae, and Nag. In the spectrum of protein Uts (Oz(−), Kern(+), Mcg(+)), the chemical shifts of His-198 C2- and C4–H proton signals are quite sensitive to the Mcg(+) substitutions. No such large shift is observed in the case of protein Kern (Oz(−), Kern(+), Mcg(−)). In protein Mae, as well as in proteins Ak, Hiro, and Jon (all of Oz(−), Kern(−), Mcg(−) isotypes), the chemical-shift difference between His-189 and His-198 C4–H proton signals is quite small in the pH range 5.3–5.7, where only an unresolved singlet can be observed (Figure 4, spectrum c). By contrast, proteins Kern and Uts, as well as protein Kob, give in the entire pH range two separate peaks for His-189 and His-198 C4–H protons; in these proteins, which have the Kern(+) marker in common at position 153, the His-189 C4–H proton signals are shifted to high field, and their chemical shifts are quite close to each other. In the case of intact λ chains, a similar but larger high-field shift was observed for the C4–H proton of His-189 of protein Kern as well as proteins Mcg, Kob, and Uts, which have the Kern(+) marker in common; the high-field shift becomes about 10 Hz in the pH range 5.5–6.5. These results clearly indicate that observation of the His-189 and His-198 signals provides a means of differentiating three types of proteins which have Kern(+) and Mcg(+), Kern(+) and Mcg(−), and Kern(−) and Mcg(−) isotypic markers.

Protein Nag (Oz(+), Kern(−), Mcg(−)) gives two separate peaks for His-189 and His-198 C4–H protons (Figure 4, spectrum d). From what has been discussed above, protein Nag can easily be differentiated from proteins with Oz(−), Kern(−), and Mcg(−) markers or those with Oz(−), Kern(+), and Mcg(+) markers. Protein Nag is different from protein Kern (Oz(−), Kern(+), Mcg(−)) in that the His-189 as well as His-198 C4–H proton signals are shifted to lower field; the

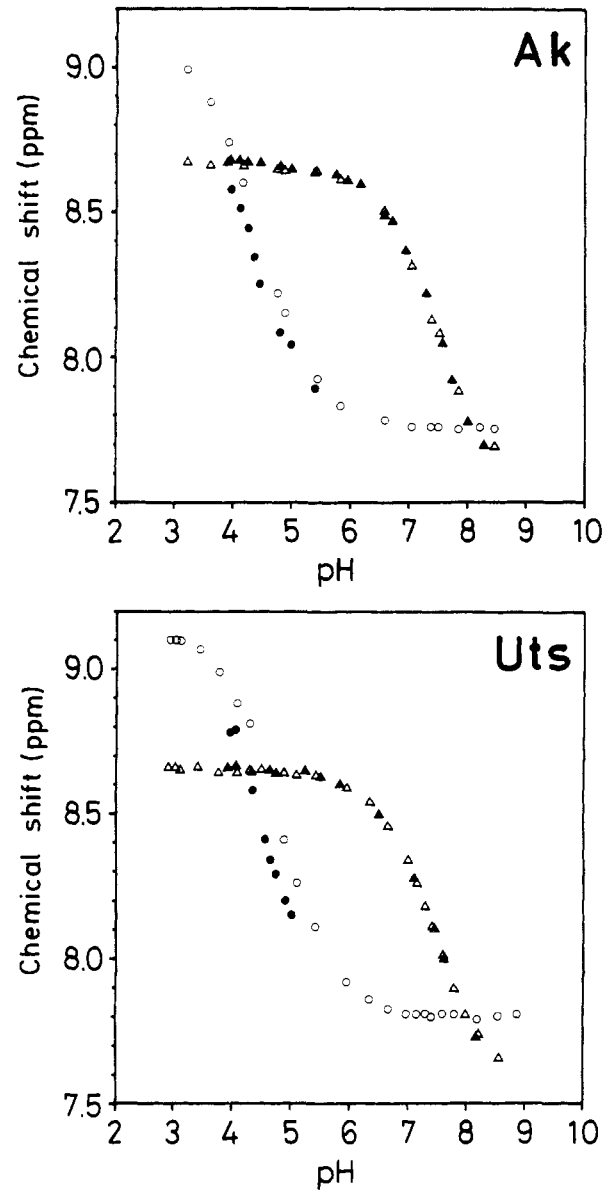

FIGURE 5: The pH dependence of the chemical shifts of the C2–H protons of His-189 and His-198 of intact λ chains Ak and Uts and of the corresponding C_L fragments. Intact λ chains: (▲) His-189; (●) His-198. C_L fragments: (△) His-189; (○) His-198.

low-field shift is significant in the pH range 5–6 where the chemical shifts change very little with pH and also below pH 4 where the chemical-shift difference for the His-189 C4–H proton of proteins Nag and Kern becomes larger than 5 Hz. In the case of C_L fragments, the line widths of the histidine signals are relatively narrow, and, therefore, the observed difference for proteins Nag and Kern well exceeds the experimental error. These results suggest that the His-189 and His-198 proton signals may also be used to identify the Oz marker.

Solution Conformation of the Constant Domain. The NMR data described so far strongly suggest that the Kern and at least one of the Mcg markers are in close spatial proximity to His-189 and His-198, respectively. On the basis of the ^{1}H NMR measurements of 15 λ-type Bence-Jones dimers, we have demonstrated that His-189 is exposed to solvent as His-105 of bovine pancreatic ribonuclease A, whereas His-198 is far less accessible to solvent (Arata & Shimizu, 1979). The pH titration curves for the C2–H proton signals of His-189 and His-198 of the intact λ chains Ak and

Uts are compared with those for the corresponding C_L fragments. As Figure 5 indicates, the His-189 and His-198 titration curves are basically similar for the intact λ chains and the corresponding C_L fragments. The C2–H proton of His-189 of all the C_L fragments examined gives quite similar titration curves which, as shown in Figure 5, also agree quite well with those for the intact λ chains. The results given in Figures 2 and 5 confirm our previous suggestion (Arata & Shimizu, 1979) that the tertiary structure of the *immunoglobulin fold* is well preserved even in the C_L fragments. In the case of the C_L fragments, the His-198 titration curves are slightly shifted to the high pH side of those for the corresponding intact λ chains. It was confirmed that two kinds of C_L fragments obtained from protein Nag, which have different N-terminal residues, i.e., leucine-104 and serine-108, give identical His-189 and His-198 titration curves. The small shifts observed for the C_L fragments and the corresponding intact λ chains may be due to the difference in conformation in the absence and presence of the covalently linked variable domain and/or to the interaction between the two constant domains in the intact λ chains; it is known that the C_L fragments exist as the monomer in solution (Karlsson et al., 1972). In the case of the C_L fragments, it was observed that protein Uts (Mcg(+)) and protein Nag (Mcg(−)) give quite similar CD spectra. This means that no major change in conformation occurs even when the Mcg(+) markers are introduced in the constant domain. However, the large shift observed for the His-198 titration curves indicates that the environment surrounding His-198 is significantly affected by the Mcg substitutions.

X-ray structural studies have shown that His-189 and His-198 are located in quite different environments (Poljak et al., 1973; Edmundson et al., 1975); His-198 is deeply buried in the *immunoglobulin fold*, where Ala-112, Ala-113, Pro-114, Phe-140, Pro-142, Ala-144, and Val-145 have atoms which are located within 4 Å from at least one of the atoms in His-198 in the constant domain of the λ chain of Fab New (Poljak et al., 1973).[4] The imidazole ring of His-198, which is most likely hydrogen bonded to the carbonyl oxygen of Pro-142 at N^{τ}, is surrounded by the hydrophobic side chains of the above amino acids residues;[5] in particular, Phe-140 C^{β} is less than 3 Å apart from His-198 C2 and N^{τ}. This is consistent with the observation by NMR that His-198 has an unusually low pK_a value and the H–D exchange at the C2 position of His-198 is extremely slow (Arata et al., 1978a; Arata & Shimizu, 1979). It should also be noted that a chain segment Ala^{112}-Ala^{113}-Pro^{114}, which contains one of the Mcg markers at position 113, is in close spatial proximity to His-198. In addition, Ala-113 and Ser-115, both of which are the Mcg markers, are quite close to Asp-139 and Ser-138, respectively, which leads to the sequence Phe^{140}-Val^{145} that is involved in close contacts with His-198. Thus, it is quite likely that the substitutions of alanine by asparagine at position 113 and serine by threonine at position 115 in the Mcg(+) proteins would alter the environment surrounding His-198 and induce the large shift in the His-198 titration curves. On the other hand, His-189 is exposed to solvent; only a small number of atoms of Ala-151, Asp-152, and Ser-153 are in close spatial proximity to His-189.[4] It is of interest, however, that the O^{γ} of Ser-153, which is the Kern marker, is only 3.3 Å apart from His-189 C2. In the Kern(+) proteins, serine at position 153 is replaced by glycine. This is very likely the reason that the Kern substitution is reflected on the His-189 titration curves. As described so far, the NMR data are quite consistent with the X-ray structure; in the intact λ chains as well as the C_L fragments in solution, the environment surrounding His-189 and His-198 appears to be quite similar to that in the X-ray structure of the constant domain of the λ chain (Poljak et al., 1973).

As Figure 2 shows, the His-189 C4–H proton signals start to shift to high field below pH 4.5. Azuma et al. (1978) have observed below pH 5 a small decrease in ellipticity at 278 nm in the CD spectrum of the C_L fragment Nag. However, in view of the fact that the high-field shift is observed only for the His-189 C4–H proton signals, it appears unlikely that the C_L fragments are subject to a major conformation change in the pH region 3.0–4.5. This point will be a subject for future study.

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[4] Atomic coordinates were obtained from the Protein Data Bank (Bernstein et al., 1977). Plotter drawings of the protein structure were made using the program STDRAW developed by Dr. Y. Mitsui and coworkers at the University of Tokyo.

[5] For the numbering of the histidyl ring, the biochemical convention is utilized here. Correspondence to the crystallographic convention is N^{π}–$N^{\delta 1}$; N^{τ}–$N^{\epsilon 2}$; C2–$C^{\epsilon 1}$; C4–$C^{\delta 2}$.

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A Common Mechanism of Hapten Binding to Immunoglobulins and Their Heterologous Chain Recombinants†

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ABSTRACT: Kinetics and thermodynamics of binding of the hapten β-D-(1–6)-galactotriose to the homogeneous IgA T-601 and to heterologous recombinants of heavy and light chains prepared from mouse myeloma IgA's X-24, J-539, and T-601, which all have the same galactan specificity, have been studied by the chemical relaxation method. All the immunoglobulin–hapten systems investigated were found to exhibit two relaxation times. The reciprocal value of the fast time increased linearly, while that of the slow time leveled off with increasing hapten concentration. This behavior indicates the presence of a fast bimolecular association and a slower monomolecular step. The data obtained for homologous and hybrid immunoglobulins were all found to fit a mechanism where the proteins exist in two conformations and hapten binding shifts their equilibrium to the higher affinity conformer. Furthermore, the kinetic and thermodynamic parameters for the hapten binding by the hybrids were found to be similar to those of their parent proteins. These results strongly suggest that this conformational transition is an inherent property of the tertiary domain structure of the antibody, probably involving changes in the interactions between heavy- and light-chain domains.

X-ray crystallographic studies of several human and murine immunoglobulins (Ig's)[1] and their fragments have shown that the chains of all these molecules are folded into a linear series of compact domains having similar tertiary structure [for reviews, see Poljak et al. (1976) and Padlan (1977)]. Each domain corresponds to one of the homology regions apparent in the primary structures of these chains. Residues involved in making both inter- and intrachain contacts between domains are highly conserved even in the variable regions and are often identical in closely related Ig's (Poljak, 1975). Thus, the mode of association between domains, including the variable ones (V_L and V_H), has been assumed to be the same or at least very similar in all antibody molecules (Padlan, 1977). This assumption has been confirmed by extensive studies where heavy and light chains, separated from their parent Ig's in dissociating solvents, were successfully reassembled to form the characteristic four-chain structure [for references, see Manjula et al. (1976) and Klein et al. (1979)]. When heterologous recombinants were similarly prepared by using heavy and light chains derived from different parent Ig's, the hybrid molecules usually exhibited a substantial reduction in hapten binding affinity and idiotypic activity as compared with those properties in their parent molecules (Manjula et al., 1976). A notable exception is a group of hybrid Ig's prepared by heterologous recombination of heavy and light chains made from the β-D-(1–6)-oligogalactan-specific homologous murine myelomas X-24, J-539, and T-601. The parent proteins are of the IgA class with κ light chains of the same variable region subgroup. The hybrids maintain an affinity for the same haptens which is comparable to that of their parent molecules (Manjula et al., 1976, 1977).

The mechanism of binding of two oligogalactose haptens to two of the above immunoglobulins, X-24 and J-539, has been examined by kinetic measurements using the chemical relaxation–temperature jump method (Vuk-Pavlović et al., 1978). This investigation produced evidence for a hapten binding induced conformational transition in both immunoglobulins. Such a hapten binding linked transition was reported previously for the poly(nitrophenyl)-specific IgA_κ secreted by plasmacytoma MOPC-460 (Lancet & Pecht, 1976).

The question arose as to whether the observed conformational transition is an inherent property of the interacting heavy and light chains. A solution might be found in the behavior of hybrid molecules produced from Ig's which have been already investigated. The present work examines the kinetics and thermodynamics of binding of β-D-(1–6)-galactotriose (Gal_3) to the homologous IgA T-601 and to the hybrid recombinants $H^{24}L^{539}$, $H^{539}L^{24}$, $H^{24}L^{601}$, and $H^{601}L^{24}$. The results show that the heterologous recombinants exhibit the same kinetic pattern of binding as their parent proteins. Thus, the conformational transitions linked to hapten binding appear to

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[1] Abbreviations used: Ig, immunoglobulin; IgA, immunoglobulin A; Fab, antigen binding fragment of Ig; Fd, NH_2-terminal half of a heavy chain; H, heavy chain of Ig; L, light chain of Ig; V, variable region; C, constant region; CDR, complementarity-determining region; FR, framework region; Gal_3, β-D-(1–6)-galactotriose; ESR, electron spin resonance; NMR, nuclear magnetic resonance.

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