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Proton Nuclear Magnetic Resonance Studies of Bence-Jones Proteins†

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ABSTRACT: ^{1}H nuclear magnetic resonance (NMR) studies of λ- and κ-type Bence-Jones proteins are reported. Fifteen λ-type Bence-Jones proteins, including proteins Blo, Mcg, Sh, Vi, and Weir, were used to examine the pH titration curves of the two constant domain histidines (His-189 and His-198). The His-189 titration curves were all quite similar, with a pK_a of 7.3 ± 0.1. On the other hand, there is significant heterogeneity in the His-198 titration curves; the pK_a values are in no case greater than 4.5. The line width of the His-198 peak is much broader than that of the His-189 peak. In addition, the C(2)–H proton of His-189 incorporates deuterium from D_2O rapidly, whereas virtually no exchange occurs at His-198. The NMR results are compared with X-ray crystallographic studies of the λ-type Bence-Jones dimer Mcg [Edmundsen, A. B., Ely, K. R., Abola, E. E., Schiffer, M., & Panagiotopoulos, N. (1975) *Biochemistry 14*, 3953] which shows that His-189 belongs to an exposed loop, whereas His-198 is oriented toward the interior. The resonances of His-189 and His-198 reflect well the environments expected from the tertiary structure, and the heterogeneity observed for the His-198 resonances probably reflects the Mcg isotypic substitutions. The constant fragment (C_L) obtained by limited tryptic digestion of the λ-type Bence-Jones protein Nag was also examined, and the results were compared with those for the intact λ-type Bence-Jones proteins. It was concluded that the tertiary structure of the immunoglobulin fold is well preserved even in the C_L fragment. ^{1}H NMR spectra of three kinds of κ-type Bence-Jones proteins were examined. There is a difference between λ- and κ-type Bence-Jones proteins in the His-189, and more significantly in the His-198, titration curves. We suggest that the difference in the chemical shift of the His-189 resonances makes it possible to quantitate the λ/κ ratio for the normal light chain. It was shown that His-198 in the κ-type Bence-Jones proteins is much more difficult to protonate; the His-198 peak begins to shift downfield only below pH 4, where the proteins begin to denature. We conclude that λ- and κ-type Bence-Jones proteins are basically similar in conformation in the constant domain. However, the constant domain of the κ-type proteins appears to be more compact than that of λ-type proteins.

Bence-Jones proteins, which are excreted into the urine of patients with multiple myeloma, are dimers of homogeneous light chains of immunoglobulins. The light chains are divided into two homology units (domains) of about 110 amino acid residues. The variable, amino-terminal domain differs markedly from one Bence-Jones protein to another, whereas the constant, carboxy-terminal domain has essentially an invariant sequence. The light chains exist in two types, κ and λ, the structural differences of which are reflected in antigenic differences.

The crystal structure of a λ-type Bence-Jones dimer designated Mcg has been analyzed at 2.3 Å by Edmundsen & co-workers (1975). X-ray studies of the dimer of the variable domain of the κ-type Bence-Jones protein REI has also been reported (Epp et al., 1974). It has been demonstrated by Edmundsen et al. (1975) that, in the Mcg dimer, each of the two light chains is different in conformation and forms an architecture which is quite similar to that of the Fab fragment of the immunoglobulin molecules; three- and four-chain layers of antiparallel β-pleated sheets constitute the basic *immunoglobulin fold*. They also reported binding studies on Mcg crystals that demonstrated that the Mcg dimer binds a variety of small molecules at three different binding sites whose structure has been elucidated in detail on the basis of the X-ray crystallographic data (Edmundsen et al., 1974).

The λ-type Bence-Jones proteins have in common two histidine residues (His-189 and His-198) in the constant domain of each of the light chain.[1] In a previous paper (Arata et al., 1978), we have reported a ^{1}H NMR spectrum of a λ-type Bence-Jones dimer Ak which contains no histidine residue in the variable domain; assignments of the C(2)–H proton signals of His-189 and His-198 have been accomplished by using a deuterium-labeling technique which is similar to that used by Markley & Kato (1975).

In the present work, we have examined by ^{1}H NMR more than 20 Bence-Jones proteins.[2] These include λ-type Bence-Jones dimers Mcg and Sh with known amino acid sequences. In addition to three Mcg(+) proteins, Mcg, Hu,

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[1] The numbering system used in the present paper is based on protein Sh (λ-type) and protein Ag (κ-type). See Putnam (1969).

[2] The authors are indebted to the following for samples of Bence-Jones proteins: Dr. T. Azuma and Dr. K. Hamaguchi, Osaka University; Dr. H. F. Deutsch, University of Wisconsin; Dr. F. W. Putnam, Indiana University; Dr. N. Hilschmann, Max-Planck-Institute; Dr. T. Ikenaka, Osaka University; Dr. S. Migita, Kanazawa University; Dr. C. Milstein, MRC Laboratory of Molecular Biology; Dr. T. Shinoda, Tokyo Metropolitan University.

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and Weir, proteins Blo, Kern, Mz, and Vi, which have known isotypic markers, were also examined. We found that the His-189 titration curves for different λ-type Bence-Jones proteins are quite similar to each other, but that there is significant heterogeneity in the His-198 titration curves. These titrations were compared with those of the constant fragment (C_L),[3] which was obtained by limited tryptic digestion of a λ-type Bence-Jones protein Nag (Azuma et al., 1978).[4] The mixed disulfide obtained from the protein Ak by partial reduction followed by alkylation was used to check whether the presence or absence of the interchain disulfide bridge can induce any change in the pK_a values of His-189 and His-198 in the constant domain. The κ-type Bence-Jones protein also has two histidine residues in the constant domain at positions which are homologous to those of the λ-type Bence-Jones proteins.[1] The pH dependence of the C(2)–H proton signals of three κ-type Bence-Jones proteins has also been followed and compared with those of the λ-type Bence-Jones proteins.

Materials and Methods

Reagents. Dithiothreitol (Sigma) and iodoacetamide (Nakarai) were reagent grade and used without further purification. Guanidine hydrochloride (Nakarai) was dissolved in 99.8% D_2O and evaporated at room temperature. This procedure was repeated three times, and guanidine deuterochloride (Gdn·DCl) thus obtained was further dried in a desiccator over P_2O_5.

Separation and Purification of the Proteins. Bence-Jones proteins Ak, Jon, Mae, and Uts (λ-type) and Ka, Mas, and Oku (κ-type) were precipitated with ammonium sulfate from the urine of patients with multiple myeloma. The proteins were purified on DEAE-cellulose with gradient elution (from 0.01 M, pH 8.4, to 0.1 M, pH 7.2, in sodium phosphate buffer). Gel filtration on a Sephadex G-100 column equilibrated with 0.1 M Tris and 0.15 M NaCl at pH 8.0 was then used, and the dimer fraction was collected. Purity of each preparation was checked by $NaDodSO_4$–polyacrylamide gel electrophoresis and immunoelectrophoresis. In addition to the above proteins, 21 λ-type Bence-Jones proteins were provided by other laboratories.[2] These include λ-type Bence-Jones dimers Mcg and Sh with the known amino acid sequence. In addition to three Mcg(+) proteins, Mcg, Hu, and Weir, proteins Blo, Kern, Mz, and Vi, which have the known isotypic markers, were also available to us.

Reduction and Alkylation of the λ-Type Bence-Jones Protein Ak. Thirty milligrams of protein Ak was dissolved in 2 mL of 0.1 M Tris and 0.15 M NaCl buffer (pH 8.0) which was 0.01 M in dithiothreitol. The solution was kept at 25 °C for 1 h. Then 2 mL of 0.022 M (10% excess) iodoacetamide was added and the solution was kept at 0 °C for 30 min. Excess reagents were removed by dialysis against deionized water, and the preparation was then lyophilized.

Bence-Jones proteins generally exist as a mixture of the disulfide-linked dimer, the noncovalent dimer, and the monomer. The ratio of the disulfide-linked dimer and the other forms of the protein was determined by $NaDodSO_4$–polyacrylamide gel electrophoresis, or by a Sephadex G-100 column equilibrated with 7% propionic acid. It was confirmed that, in the preparation of protein Ak used in the present experiment, more than 95% of the light chain exists as the covalent dimer. On the other hand, no detectable amount of the covalent dimer was found in the preparation of κ-type Bence-Jones protein Mas. It has been checked that reduced and alkylated protein Ak (hereafter referred to as the mixed disulfide) does not contain any detectable amount of the covalent dimer.

Refolding of the λ-Type Bence-Jones Protein Ak Which Is Unfolded by 4 M Gdn·DCl. Twenty milligrams of protein Ak was dissolved in 0.5 mL of 4 M Gdn·DCl in D_2O at pH 5.0, and incubated at 37 °C for 24 h. The resultant solution was then dialyzed at 5 °C for 48 h against three changes of 50 mL of D_2O and lyophilized.

NMR Measurements. Ten milligrams of each Bence-Jones protein was dissolved in 0.3 mL of 0.2 M $NaCl/D_2O$. In the case of the C_L fragment of protein Nag,[4] 5 mg of the protein was dissolved in 0.3 mL of 0.2 M $NaCl/D_2O$. When the sample solution was incubated at 30 °C, pH 8.0–8.5, NH proton signals in the aromatic region gradually disappeared. 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. NMR spectra were obtained at 100 MHz with a JEOL PS-100 spectrometer in the correlation mode (Arata & Ozawa, 1976). Typically 2000 transients (512 Hz/0.5 s) were accumulated to improve the signal-to-noise ratio. All chemical shifts are given in ppm from external DSS (5% in D_2O). The probe temperature was 29 °C throughout the experiment.

Results

Figure 1 shows the aromatic region of the ^1H NMR spectrum of protein Ak which contains two histidine residues in the constant domain, but none in the variable domain. When protein Ak is dissolved in D_2O at neutral pH, two peaks 1 and 2 are observed on the shoulder of a large and very broad peak as shown in Figure 1A. The broad signal is observed in common in all λ- as well as κ-type Bence-Jones proteins examined in the present study. This peak does not titrate with pH and gradually decreases in intensity when the protein is incubated in D_2O at 30 °C above pH 8 (see Figure 1B). The two peaks 1 and 2 titrate with pH. As Figure 2 shows, a large titration shift of peak 2 is observed below pH 5. The titration curves of these two peaks are given in Figure 3. From the chemical shifts, these titrating peaks are most likely due to the C(2)–H proton of the two histidine residues in the constant domain of the Bence-Jones protein (Roberts & Jardetzky, 1970; Markley, 1975). In order to confirm that the peaks 1 and 2 are actually due to the two histidine residues in the constant domain, protein Ak was unfolded in 4 M Gdn·DCl in D_2O at pH 5.0, and then refolded to the native form by dialysing the solution against D_2O. The protein Ak thus obtained gives a ^1H NMR spectrum, which is shown in Figure 1C. The peaks 1 and 2 are not altered, whereas the broad peak clearly disappears. As shown in Figure 3, the titration curves for peak 1 and peak 2 of the refolded protein are virtually identical with those of the native protein Ak. It has also been confirmed that the CD spectrum of the refolded protein Ak is virtually identical with that of the native protein. These results indicate that the refolding is completely reversible under the experimental conditions employed. At the same time, this experiment confirms that the peaks 1 and 2 in Figures 1 and 2 are actually due to the C(2)–H proton of the histidine residues of the constant domain. As reported previously (Arata et al., 1978), a deuterium-labeling technique has been used to assign peaks 1 and 2 to His-189 and His-198, respectively. Further support for this assignment comes from an experiment

[3] Abbreviations used: C_L, the constant fragment of the light chain; DSS, 2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt; Gdn·DCl, guanidine deuterochloride.

[4] The C_L fragment of protein Nag is a generous gift from Dr. T. Azuma and Dr. K. Hamaguchi of Osaka University.

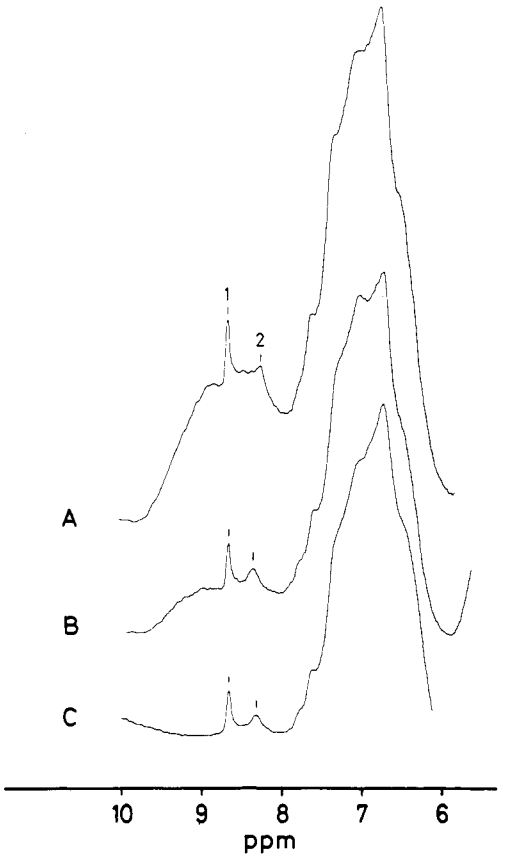

FIGURE 1: The 100-MHz ^{1}H NMR spectra of the aromatic region of λ-type Bence-Jones protein Ak. (A) Dissolved in 0.3 mL of 0.2 M NaCl/D_2O and measured at pH 4.47. (B) Incubated in 0.3 mL of 0.2 M NaCl/D_2O at pH 8.5, 30 °C for 2 h, and measured at pH 4.30. (C) Unfolded in 4 M Gdn·DCl at pH 5.0, and then refolded by dialyzing against D_2O. After lyophilization, the refolded protein was dissolved in 0.3 mL of 0.2 M NaCl/D_2O and measured at pH 4.38. Ten milligrams of protein Ak was used for each measurement. The probe temperature was 29 °C. Chemical shifts are in ppm from external DSS (5% in D_2O).

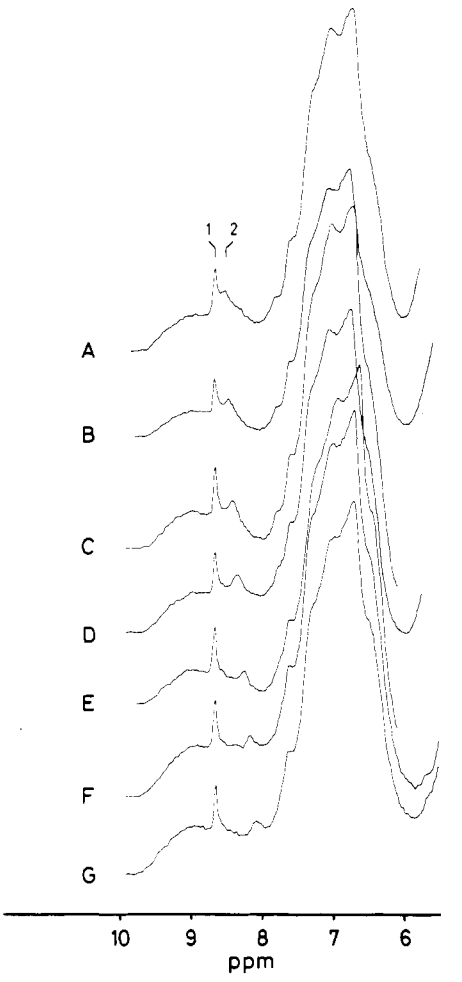

FIGURE 2: The 100-MHz ^{1}H NMR spectra of the aromatic region of λ-type Bence-Jones protein Ak at various pH values. pH: (A) 4.02, (B) 4.12, (C) 4.20, (D) 4.30, (E) 4.51, (F) 4.63, (G) 4.76. Ten milligrams of protein Ak was dissolved in 0.3 mL of 0.2 M NaCl/D_2O and preincubated at pH 8.5 and 30 °C for 2 h. The probe temperature was 29 °C. Chemical shifts are in ppm from external DSS (5% in D_2O).

with the constant fragment (C_L) of λ-type Bence-Jones protein Nag. As shown in Figures 4 and 5, the C_L fragment which is known to exist as the monomer (Karlsson et al., 1972) gives in the whole pH range 3–9 two peaks whose chemical shifts are quite similar to those of the intact protein. This point will be discussed later. It has also been confirmed by the above-mentioned experiment that the large broad peak is due to the exchangeable protons which are probably for the most part those of the backbone NH groups.

The aromatic region of a ^{1}H NMR spectrum of the C_L fragment of the λ-light chain obtained by limited tryptic digestion of the λ-type Bence-Jones protein Nag (Azuma et al., 1978) is given in Figure 4C. There are at least four peaks which titrate with pH. The titration curves of these four peaks are illustrated in Figure 5. The pK_a for peaks 1 and 2 of the C_L fragment are 7.3 and 4.3, respectively. Comparison of the titration data shown in Figure 5 with those in Figure 3 leads to the conclusion that peaks 1 and 2 observed in the C_L fragment are due to the C(2)–H proton of His-189 and His-198, respectively. The pK_a values of peaks 3 and 4 are quite similar to those of peaks 2 and 1, respectively. These results indicate that peaks 3 and 4 are due to the C(4)–H proton of His-198 and His-189, respectively.

As Figures 6A and 6B show, all λ-type Bence-Jones proteins examined in the present work have the His-189 and His-198 peaks in common. Of the two histidine peaks, the titration curves for His-189 are in close agreement with each other. The

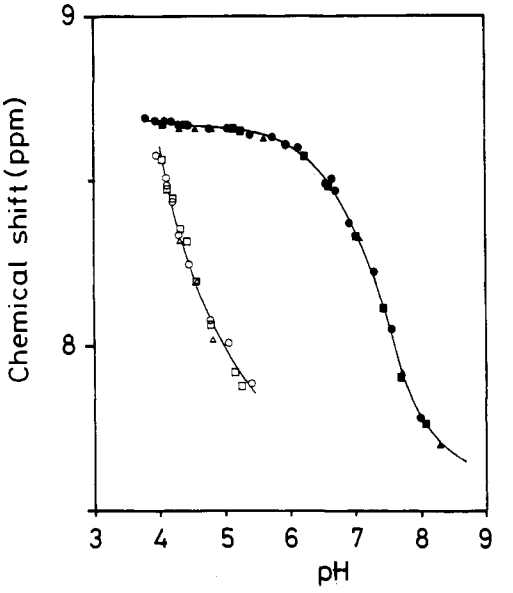

FIGURE 3: The pH dependence of the chemical shifts of the peaks 1 and 2 of λ-type Bence-Jones protein Ak. μ = 0.2, 29 °C. Native protein Ak: peak 1 (●) and peak 2 (○); unfolded in 4 M Gdn·DCl at pH 5.0 and then refolded, peak 1 (▲) and peak 2 (△). The mixed disulfide: peak 1 (■) and peak 2 (□).

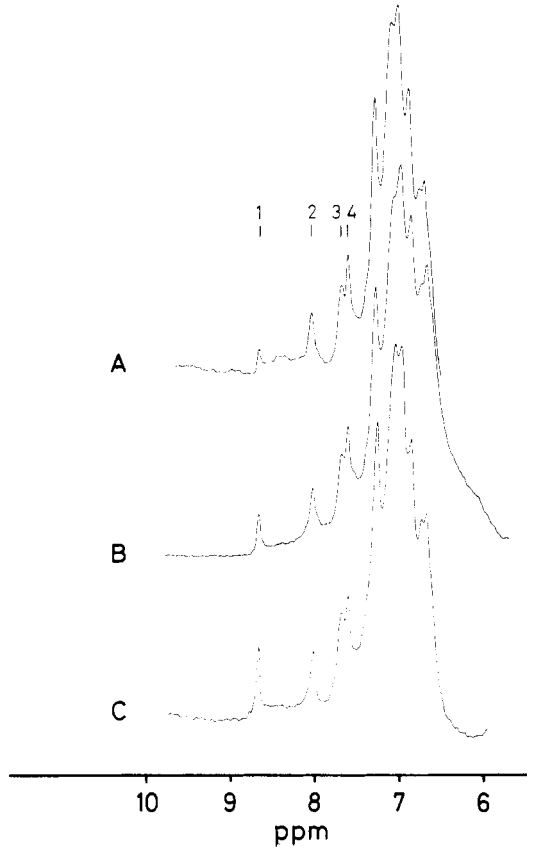

FIGURE 4: The 100-MHz ^1H NMR spectra of the aromatic region of the C_L fragment of λ-type Bence-Jones protein Nag. (A) Incubated for 38 h at pH 8.5 and 37 °C and measured at pH 5.04; (B) incubated for 14 h at pH 8.5 and 37 °C and measured at pH 5.06; (C) intact C_L fragment, measured at pH 5.02. For each measurement, 5 mg of the C_L fragment was dissolved in 0.3 mL of 0.2 M NaCl/D_2O. The probe temperature was 29 °C. Chemical shifts are in ppm from external DSS (5% in D_2O).

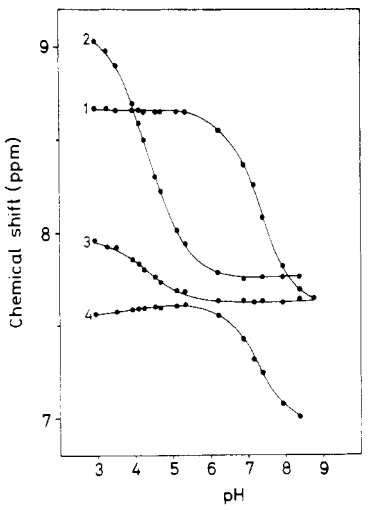

FIGURE 5: Titration curves of the histidine C2- and C4-H proton peaks of the C_L fragment of the λ-type Bence-Jones protein Nag. $\mu = 0.2$, 29 °C. Assignments: (1) C2-H (His-189); (2) C2-H (His-198); (3) C4-H (His-198); (4) C4-H (His-189).

His-189 resonances of all λ-type Bence-Jones proteins examined can be represented by a sigmoidal curve with a pK_a of 7.3 ± 0.1. On the other hand, the His-198 titration shows a significant degree of diversity (see Figure 6B). In most cases, the Bence-Jones proteins become denatured below pH 4. Therefore, it is generally difficult to determine for each of the proteins the pK_a value for His-198 accurately. Still it is quite certain that the pK_a value for His-198 is not greater than 4.5.

Results have been obtained for several histidine residues in the variable domain as well. Their pK_a values, shown in Figure 6C, are quite different from each other. In the case of the λ chains studied so far, all histidine residues in the variable domain give pK_a values which are lower than that of His-189, but significantly higher than that of His-198; most give pK_a values that are closer to that of the His-189 peak.

The κ-type light chain also has two histidine residues in the constant domain at homologous positions (His-189 and His-198).[1] Three κ-type Bence-Jones proteins, Ka, Mas, and Oku, were used in the present study. Proteins Ka and Oku have no additional histidine in the variable domain, whereas protein Mas has one histidine residue. Examples of the ^1H NMR spectra of κ-type proteins Ka and Mas are shown in Figure 7. By analogy with the λ-type light chain, peaks 1 and 2 can be assigned to His-189 and His-198, respectively. This assignment was confirmed by an experiment similar to that used to make assignments of the two constant domain histidine residues in the λ-type Bence-Jones protein Ak. As Figure 7 shows, His-189 gives a sharp signal, which results in the titration curves as illustrated in Figure 8. A pK_a value of 7.4 is obtained which is similar to but not identical with the value 7.3 of the λ-type Bence-Jones proteins given previously. The titration curve itself is also similar to that of the λ-type Bence-Jones proteins. On the other hand, His-198 does not appear to titrate even if the pH is decreased to 4. It begins to shift downfield only below pH 4, where denaturation appears to be significant, preventing further experiments in this pH region.

Discussion

In all λ-type Bence-Jones proteins examined, the line width of the His-198 peak is much broader than that of the His-189 peak. This suggests that, in the Bence-Jones proteins, His-198 is less mobile than His-189. The pK_a values of His-189 for all λ-type Bence-Jones proteins examined are 7.3 ± 0.1. By contrast, the pK_a values of His-198 are unusually low. Although it is virtually impossible to determine the pK_a values of His-198 accurately from the fragmentary titration data given in Figure 6B, it is quite certain that the pK_a values are in no case greater than 4.5. In addition, there is a large difference in the rate at which the C(2)–H protons of His-189 and His-198 incorporate deuterium from the solvent D_2O. In the case of protein Ak, at pH 8.5, the half-time for His-189 is less than 20 h, whereas more than 95% of proton at the C(2)–H position of His-198 is retained after an incubation of 24 h (Arata et al., 1978). Using proteins some of which have known crystallographic structures, Minamino et al. have shown that plots of the second-order rate constant for the incorporation of tritium at the C(2)–H position vs. pK_a for the corresponding histidine residue can be a measure of accessibility of each histidine residue to solvent (N. Minamino, H. Matsuo, and K. Narita, unpublished results). In view of their analysis, we conclude that His-189 of λ-type Bence-Jones proteins is as exposed to solvent as is His-105 of bovine pancreatic ribonuclease A. On the other hand, His-198 is far less accessible to solvent from the above criterion.

The above results are consistent with the X-ray crystallographic studies of the λ-type Bence-Jones dimer Mcg which show that His-189 belongs to a loop connecting two antiparallel segments, one in the three-chain layer and the other in the four-chain layer, and is fully exposed to solvent, whereas His-198 is located in an antiparallel segment in the three-chain layer, and is oriented toward the interior of the domain (Edmundsen et al., 1975). In view of these results, it is quite

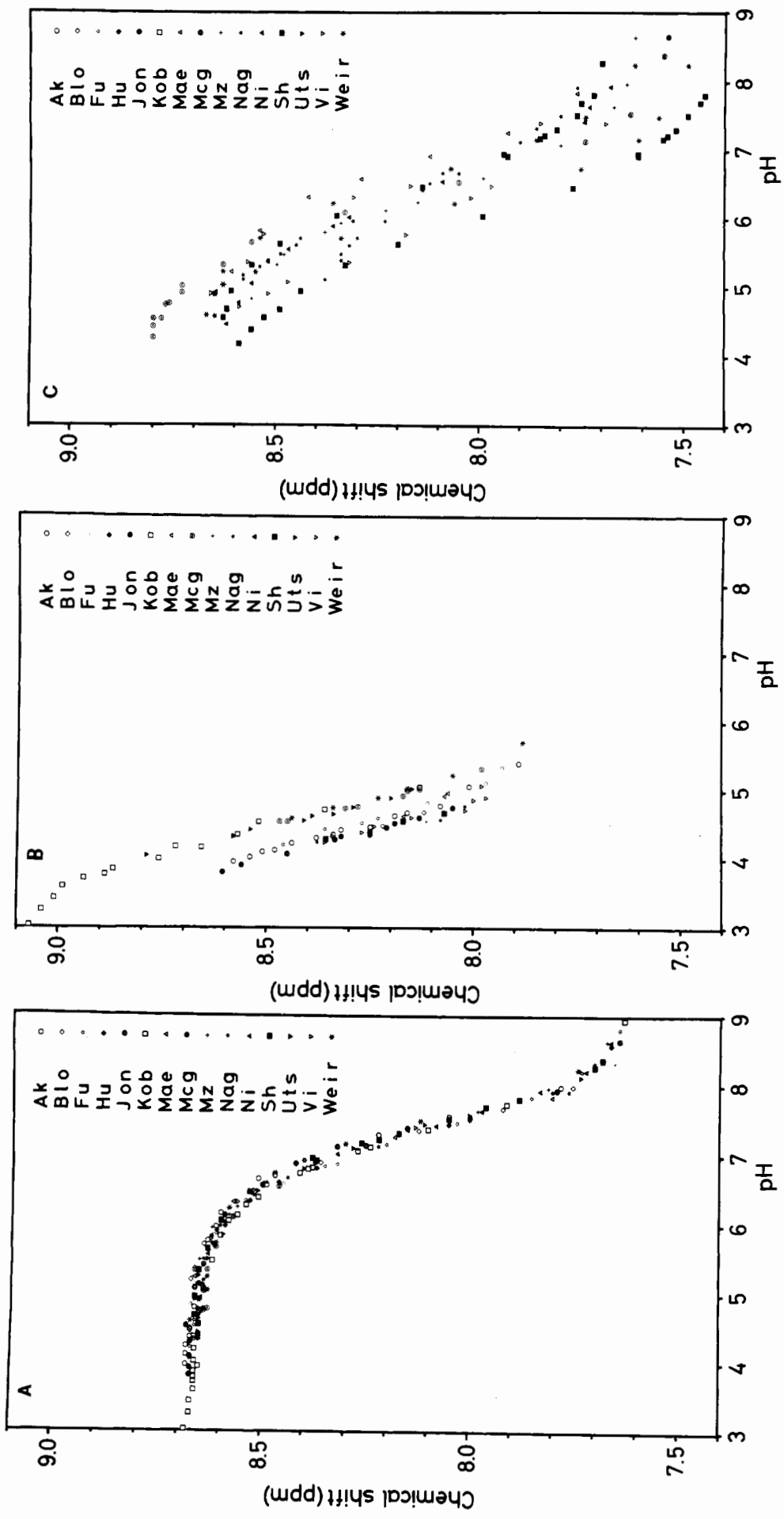

FIGURE 6: The pH dependence of the chemical shifts of the histidine C2-H proton peaks of 15 λ-type Bence-Jones proteins. Protein concentration: 10 mg in 0.3 mL of 0.2 M NaCl/D_2O. The probe temperature was 29 °C. Chemical shifts are in ppm from external DSS (5% in D_2O). (A) His-189; (B) His-198; (C) histidines in the variable domain.

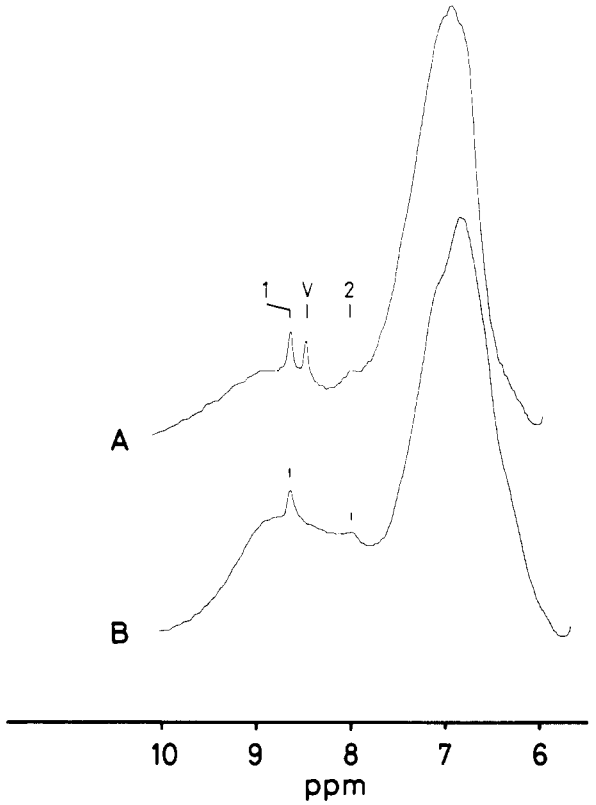

FIGURE 7: The 100-MHz ^1H NMR spectra of the aromatic region of κ-type Bence-Jones proteins (A) Mas and (B) Ka. Peaks 1 and 2 are due to His-189 and His-198, respectively. The peak designated V in the spectrum of protein Mas is due to histidine which exists in the variable domain. Protein concentration: 10 mg in 0.3 mL of 0.2 M NaCl/D_2O. The probe temperature was 29 °C. Chemical shifts are in ppm from external DSS (5% in D_2O).

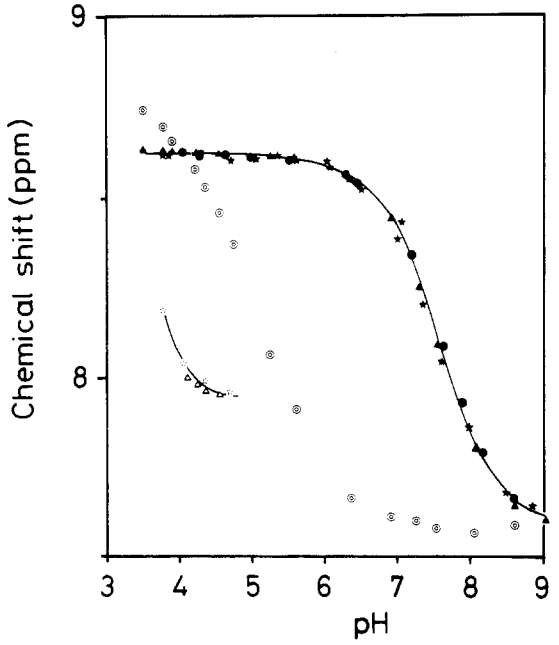

FIGURE 8: Titration curves of the histidine peaks of the κ-type Bence-Jones proteins Mas, Ka, and Oku. μ = 0.2, 29 °C. His-189: Oku (●), Ka (★), Mas (▲). His-198: Ka (☆), Mas (△). Histidine in the variable domain: Mas (◎).

conceivable that His-198, which is located in the interior of the immunoglobulin fold, would be much less favored energetically when the imidazole ring is protonated. It is most likely for this reason that His-198 has such an unusually low pK_a value. In the above sense, the His-189 and His-198 peaks may be said to reflect the tertiary structure quite well which is characteristic of the immunoglobulin fold.

It should be noted that His-189 and His-198 of the C_L fragment show the titration behavior which is basically the same as that for the intact Bence-Jones proteins. Unfortunately, the His-198 peak of the intact protein Nag was not obtainable below pH 5, where the protein appears to be denatured. Therefore, it was not possible to compare in detail the His-198 titration curve of the intact Bence-Jones protein and its C_L fragment. An attempt is now being made to obtain C_L fragments from other λ-type Bence-Jones proteins. The titration curves for His-189 observed in the intact protein and the C_L fragment are virtually identical. As in the case of the intact proteins, the His-198 peak of the C_L fragment also gives a quite low pK_a of 4.3. The rate at which the C(2)–H proton of the two histidine residues in the C_L fragment incorporates deuterium from the solvent D_2O was determined in the same way as for the case of the intact protein Ak. The results are reproduced in Figure 4. Just as in the case of the intact protein Ak, His-189 incorporates deuterium quite rapidly, whereas virtually no exchange occurs at His-198. The half-time for His-189 of the C_L fragment is quite similar to that of the intact protein Ak. These results strongly suggest that the tertiary structure of the immunoglobulin fold is well preserved even in the C_L fragment which is known to exist as the monomer in solution (Karlsson et al., 1972). As Figure 4 shows, resolution of the ^1H NMR spectrum of the C_L fragment is much better than that of the intact Bence-Jones dimer, and C(4)–H as well as C(2)–H proton peaks can be clearly observed, presumably because of the low molecular weight of the C_L fragment and, therefore, more rapid tumbling. However, it should be noted that the line width of the His-198 peak of the C_L fragment is much narrower than expected from the difference in line width for the His-189 peaks in the intact dimer and the C_L fragment. This suggest that in the C_L fragment His-198 has more freedom of internal motion than in the intact dimer. Under the experimental conditions used in the present study, virtually no exchange occurs at the C(2)–H position of His-198 in both the intact dimer and the C_L fragment. Therefore, it was not possible to compare the relative degree of burial of His-198 in these two proteins.

As presented in Figure 6B, the His-198 titration curves for the intact Bence-Jones dimers examined show a high degree of heterogeneity. The His-198 chemical shifts for proteins Mcg, Weir, Uts, and Kob (group A) are different from the rest of the proteins (group B). A simple explanation is to attribute the difference solely to that of the pK_a values for the two groups. However, this difference could also result from chemical-shift changes in the neutral and positively charged forms of the imidazole rings for these two groups. In order to further confirm the existence of heterogeneity in the His-198 titration curves, a 1:1 mixture of protein Ak (group B) and protein Kob (group A) was examined. The mixture gave two peaks which correspond to the His-198 chemical shifts given in Figure 6B for each of these proteins, whereas His-189 peak was a singlet with narrow line width. On the other hand, a 1:1 mixture of protein Ak (group B) and protein Fu (group B) gave a singlet peak even for His-198, the line width of which was quite similar to that of each of the proteins Ak and Fu. These results clearly show that the constant domain of the λ-type light chain is not quite constant as far as the ^1H NMR of His-198 is concerned. It was confirmed that, in the preparation of protein Ak used in the present experiment, more than 95% of the light chain exists as the covalent dimer. The

interchain disulfide bridge of protein Ak was reduced and alkylated by using iodoacetamide. The titration curves for His-189 and His-198 for the mixed disulfide thus prepared are included in Figure 3. As far as the titration curves for His-189 and His-198 are concerned, there is very little difference between the native protein and the mixed disulfide. In view of the results of these experiments, it may be concluded that the difference in ratio of the covalent and noncovalent dimers in different preparations of λ-type Bence-Jones proteins would not influence the titration curve for His-198 to any observable extent. A slight difference in shape of the aromatic envelope is noticed in the native protein and the mixed disulfide. However, in view of the limited resolution attainable at 100 MHz, no further discussion will be made. The difference observed in the shape of the aromatic envelope is, however, consistent with CD studies reported by Azuma et al. (1978), who have shown that there is a difference in conformation between the covalent and noncovalent dimers.

Another possibility for the observed heterogeneity is the effect of the variable domain on the chemical shift of the His-198 peak. This would mean that the different subgroups of the variable domain correspond to different His-198 titration curves. It has been demonstrated that variable segments from different subgroups of the same type differ in about 25 to 35 residues of their approximmtely 110 residues; however, variable segments that belong to the same subgroup still differ in about 10 to 15 residues (Eisen, 1973).

As Figure 6B shows, a clear and distinct difference exists in the His-198 titration curves for the four proteins Mcg, Weir, Uts, and Kob and the remaining proteins. In view of these results, it is unlikely that the heterogeneity as observed in the His-198 titration is governed by the variable domain of the light chain. A most likely and intriguing possibility is that the heterogeneity as observed through the His-198 resonance is due primarily to the amino acid substitutions in the constant domain.

The well-known Kern, Oz, Mz, and Mcg markers as well as other amino acid substitutions have so far been reported (Lieu et al., 1977). Among the λ-type Bence-Jones proteins examined in the present work, proteins Mcg, Weir, Uts, and Kob (group A) give the His-198 titration curves which are significantly different from the rest of the proteins (group B). See Figure 6B. Among proteins in group A, protein Mcg has of course the Mcg substitutions in the constant domain (Fett & Deutsch, 1974, 1975). Protein Weir has been shown to be of the Mcg (+) type with an additional substitution at 157 (Fett & Deutsch, 1976). Protein Hu, which has also been identified as being of the Mcg type (Lieu et al., 1977), was also checked, but this protein apparently becomes denatured below pH 5.3, and therefore the relevant part of the titration curve is not observable. Among proteins in group B are: protein Sh (Wikler et al., 1967; Putnam, 1969) and protein Vi (Fett & Deutsch, 1975) which have been shown to be Mcg (−), Oz(−), Kern(−), and Mz(−); protein Blo (Lieu et al., 1977) with the Oz(+) (Ein & Fahey, 1967; Ein, 1968), as well as Mcg (−), Kern(−), and Mz(−) markers; and protein Ak which is Oz(−) (Arata et al., 1978). Protein Kern (Ponstingl & Hilschmann, 1969; Hess et al., 1971) was also available to us, but the preparation we used was not quite satisfactory for the study of the tertiary structure of the protein. An effort is now being devoted to obtain a preparation more suitable for this purpose. It should be noted that Mcg(+) proteins have the Kern marker (Fett & Deutsch, 1974, 1975). Unfortunately, protein Mz (Milstein, 1967; Milstein et al., 1967) does not give the His-198 peak below pH 5, where it apparently becomes denatured. However, the Mz(+) substitutions have so far been found in only one instance (Lieu et al., 1977).

In view of these results, it now appears to be quite likely that the His-198 titration curves observed for proteins Mcg, Weir, Uts, and Kob are characteristic of the Mcg isotype of the λ-type light chain.

In the Mcg light chain, alanine is replaced by asparagine at 113, serine by threonine at 115, serine by glycine at 153, and threonine by lysine at 164 (Fett & Deutsch, 1974, 1975).[1] According to the X-ray crystallographic data of Edmundsen et al. (1975), positions 113 and 115 where two of the four Mcg substitutions occur are located in an antiparallel segment in the four-chain layer; they are spatially very close to His-198, which belongs to the middle segment in the three-chain layer and is oriented toward the interior of the immunoglobulin fold. This is most probably the reason that the Mcg substitutions induce such a large shift of the His-198 titration curves. Protein Weir has been shown to have an additional amino acid substitution at 157 where lysine is replaced by glutamic acid (Fett & Deutsch, 1976). The X-ray crystallographic studies by Edmundsen et al. (1975) have demonstrated that the Kern and Oz markers as well as the additional substitution in protein Weir at 157 are located in exposed loops which are far apart from His-198. Therefore, it is unlikely that the existence of these substitutions can exert a significant influence on the His-198 titration curve.

It is difficult to detect the amino acid substitutions in the constant domain by traditional methods such as electrophoresis and CD because the structure of the variable domain is much more heterogeneous than that of the constant domain. It is well-established that the Kern and Oz markers can be detected serologically (Solomon, 1976). This implies that loci at which the amino acid substitutions occur in these types of proteins are at the surface of the molecules and exposed to solvent (Reichlin, 1975). The X-ray crystallographic studies by Edmundsen et al. (1975) demonstrated that the Kern and Oz markers are actually located in loops connecting two antiparallel segments and are fully exposed to solvent. As far as we know, no successful use of the serological method has been reported so far in detecting the Mcg isotype. As demonstrated in the present work, ^{1}H NMR peaks of the histidine residues in the constant and variable domains can be observed separately, and observation of the His-198 peak can be a rapid means of identifying the Mcg proteins which are difficult to detect serologically. There is a small but significant difference in the titration curves for His-189 of λ- and κ-type Bence-Jones proteins. Preliminary work performed so far by using the light chain separated from the normal human IgG suggested that, in the pH range 7–8, the chemical-shift difference between the His-189 peaks of λ- and κ-type light chains is large enough even at 100 MHz so that measurements of the intensity of these two peaks may be used to quantitate the λ/κ ratio for the normal light chain.

It should also be noted that there is a larger difference between the His-198 titration curves for the λ- and κ-type Bence-Jones proteins examined. In the case of the κ-type Bence-Jones proteins, the His-198 peak is far more difficult to observe than that of the λ-type proteins; the His-198 peak is much broader than that of the λ-type proteins and begins to shift downfield only when the pH becomes lower than 4, where the proteins start to unfold and give very broad peaks, probably due to the formation of aggregates.

In view of the results mentioned above, it may be concluded that λ- and κ-type Bence-Jones proteins are basically similar in conformation in the constant domain. However, it appears

that, in the κ-type proteins, where His-198 is more difficult to protonate, the immunoglobulin fold is more compact than in the case of the λ-type proteins.

X-ray crystallographic studies of the Mcg dimer (Edmundsen et al., 1975) have revealed that the spatial relations between the constant and variable domains in the two light chains are markedly different. By contrast, the two histidines at the identical position in the constant domains of the two light chains as well as those that are observable in the variable domains give identical chemical shifts even at 360 MHz,[5] indicating that, as far as the environment surrounding the histidine residues is concerned, the conformations of constant and variable domains are quite similar for the two monomeric units of the dimer in solution.

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Shapes of Proteins L1, L9, L25, and L30 from the 50S Subunit of the *Escherichia coli* Ribosome, Determined by Hydrodynamic Studies†

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ABSTRACT: Proteins L1, L9, L25, and L30, purified by a nondenaturing method from the 50S ribosomal subunit of *Escherichia coli* A19, have been characterized. The four proteins were studied under conditions which resemble those used for reconstitution experiments. These proteins have $s^0_{20,w}$ values of 2.0 S, 1.8 S, 1.8 S, and 1.0 S and $D_{20,w}$ values of 8.4 × 10^{-7}, 9.0 × 10^{-7}, 14.0 × 10^{-7}, and 15.0 × 10^{-7} cm^2/s. Apparent specific volumes at 20 °C are 0.738, 0.733, 0.700, and 0.735 mL/g for the four proteins. The respective molecular weights determined by sedimentation equilibrium are 25 000, 17 300, 12 000, and 6500. The intrinsic viscosity values for the four proteins are 4.0, 5.5, 3.6, and 3.2 mL/g. From these hydrodynamic parameters L1 and L9 appear to have globular or at most only slightly elongated shapes, whereas L25 and L30 appear to be definitely globular.

Many innovative studies have been reported in the literature in an attempt to understand the structure and function of ribosomes. For better understanding of protein–protein and protein–RNA interactions in ribosomes, the shapes of several ribosomal proteins have been determined in situ, on the surface of the ribosome, and in solution. The elucidation of a number of specific antibody binding sites for individual proteins on the ribosome using immune electron microscopy techniques has given evidence that some of the protein molecules may have

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