CHEMISTRY LETTERS, pp. 283-286, 1982.

ESR MEASUREMENTS OF INTERACTION BETWEEN Mn^{2+} AND PROTEINS. A NEW METHOD FOR ANALYSIS OF EXPOSED CHARGED RESIDUES

Shoko NISHIHARA, Tokuko WATANABE, and Yoji ARATA*,1)
Department of Chemistry, Faculty of Science, The University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113

A new method, which is based on the use of Mn^{2+} ion as an ESR probe, has been developed to examine the number of charged residues on the surface of protein. Results obtained using this method will be briefly discussed for several kinds of proteins.

In biological interactions involving proteins, the distribution of charged residues on the surface of the protein molecule is one of key factors. For example, it has recently been suggested that exposed charged groups are responsible for the binding of the complement to the antibody.[2,3,4] Potentiometric titration that has commonly been used to quantitate the charged groups is not quite versatile in dealing with a variety of systems extensively. In this note, we report a new method which uses Mn^{2+} ion as an ESR probe for the analysis of the distribution of charged residues on the surface of protein.

Mn^{2+} ion in aqueous solution gives a well-resolved sextet ESR signal due to hyperfine interaction. It has been shown that formation of complexes with lower symmetry results in severe broadening due to zero field splitting and makes the ESR signal undetectable.[5,6] This means that, in the solution of a protein with excess Mn^{2+} ions added, the concentration of Mn^{2+} ion bound to the protein can be estimated from the ESR signal that is solely due to the free Mn^{2+} ion. In proteins, possible sites of interaction with Mn^{2+} ion are in the pH range 2 - 8 aspartic acid (pK_a = 3.7), glutamic acid (pK_a = 4.3), and histidine (pK_a = 6.0) residues; the pK_a values given in the parentheses are those of the monomeric amino acids. At pH higher than pK_a, each of these residues would be deprotonated and becomes available for interaction with Mn^{2+} ion.

We first confirmed by ^1H NMR measurements that only exposed residues are responsible for the Mn^{2+}-protein interaction. The Bence-Jones protein, which is

the dimer of homogeneous light chains of immunoglobulins, has two histidine residues (His-189 and His-198) in the constant domain.[7] It has been shown that His-189 (pK_a = 7.3) is exposed to solvent, whereas His-198 ($pK_a \leq 4.5$) is buried in a hydrophobic region.[8,9] Figure 1 shows the pH dependence of the ^1H NMR linewidth for the C2-H proton of the two histidines in the presence of Mn^{2+} ion. Above pH 6, the His-189 peak showed a significant broadening due to the binding of Mn^{2+} ion. By contrast, the His-198 peak was not broadened even at pH 7 which is higher than the pK_a of His-198. This indicates that only the exposed His-189 residue can interact with Mn^{2+} ion. A similar result was obtained for an exposed histidine residue which exists in the hinge region of human IgG 1 proteins.[10]

In Figure 2, an example of the pH dependence of the concentration of the free Mn^{2+} ion is given for human normal IgG protein; 5 mg of the protein was used for the experiment. The observed pH dependence can be used to determine the relation

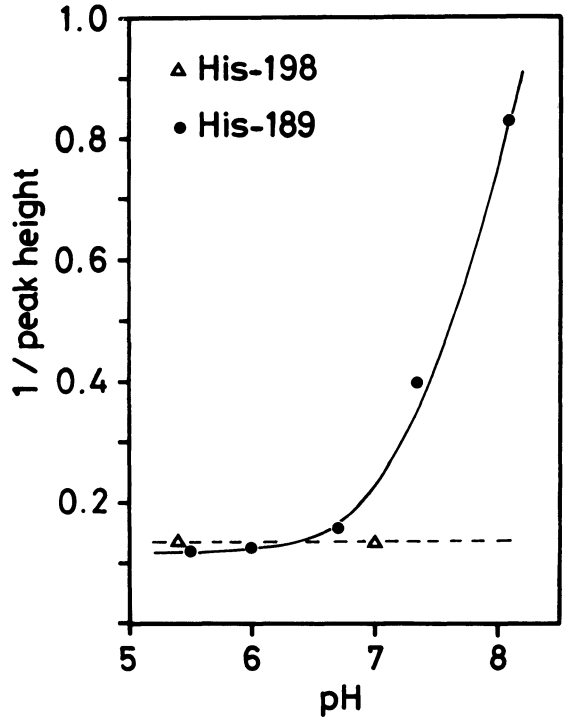

Figure 1. Paramagnetic broadening of histidine peaks (C2-H proton) in the constant fragment of Bence-Jones protein (Ak).

1.5 mM constant fragment and 0.05 mM $MnCl_2$ in 0.2 M NaCl / D_2O solution.

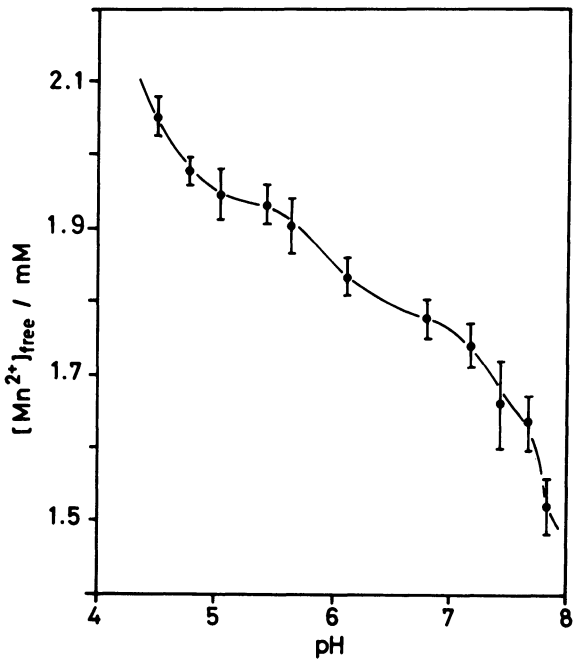

Figure 2. The pH dependence of the concentration of free Mn^{2+} ion in an aqueous solution of human IgG.

0.01 mM normal human IgG and 2 mM $MnCl_2$ in 0.2 M NaCl aqueous solution.

between an apparent binding constant K and the number of binding sites n, where K represents an average for the n binding sites. We assume that K is given by

$$K = \frac{[Mn\text{-}P]}{[Mn^{2+}]\ [P]}$$

where $[Mn^{2+}]$ and [Mn-P], which are the concentrations of the free Mn^{2+} ion and the Mn^{2+}-protein complex, respectively, can be determined by

$$[Mn^{2+}] = [Mn^{2+}]_{pH8.0}$$

$$[Mn\text{-}P] = [Mn^{2+}]_{pH4.5} - [Mn^{2+}]_{pH8.0} .$$

[P] represents the total number of Mn^{2+} ion binding sites in solution and is given by

$$[P] = n\ [Protein] - [Mn\text{-}P].$$

The concentrations of free Mn^{2+} ion at pH 8.0 and 4.5 were estimated from the intensity of ESR signals.[11]

Similar experiments were performed using ribonuclease A and Bence-Jones protein where no preferential sites are known to exist for Mn^{2+} ion with large binding constants. The relations between K and n obtained for these two proteins are summarized in Table 1. The numbers of the binding sites in these proteins were estimated by using the known potentiometric titration data[12,13] and were used to calculate the binding constants, which are also included in Table 1. Although the function and the properties of each of these proteins are different, the binding constants are quite similar to each other. On the basis of these results, it may be concluded that the value K = 8 x 10 can be accepted as representing the average binding constant for the weak interaction of proteins with Mn^{2+} ion.

The relation between K and n for rabbit IgG is shown in Table 1. The number

Table 1. The apparent binding constant, K, and the number of binding sites, n, of proteins to Mn^{2+} ion (pH 4.5 - 8.0).

Proteins	Relation between K and n	n	K
Ribonuclease A[a]	$K = 4.8 \times 10^2 / (n - 1)$	7	8.0×10
Bence-Jones protein (Ak)[a]	$K = 6.2 \times 10^2 / (n - 1.2)$	10	7.1×10
Rabbit IgG[b]	$K = 3.0 \times 10^3 / (n - 6)$	4×10	8×10

2 mM $MnCl_2$ in 0.2 M NaCl aqueous solution. Protein concentrations: 0.1 mM[a] and 0.05 mM[b].

of binding sites, n, calculated by using the above-mentioned value, K = 8 x 10, is 4 x 10. This result is in good agreement with previously reported values, 40 and 48, which were estimated by potentiometric titration.[14,15] We thus conclude that the Mn^{2+}-probe ESR method described above should be useful to quantitate exposed charged residues on the surface of protein where no preferential binding sites exist for metal ion. In this case, this method can be an alternative to potentiometric titration and is far more convenient especially in dealing with a limited amount of samples.

We are grateful to Professor S. Fujiwara for his interest and encouragement.

References

1) Present Address: Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113.

2) F. Vivanco-Martinez, R. Bragado, J. P. Albar, C. Juarenz, and F. Ortizmasllorens, Mol. Immunol., 17, 327 (1980).

3) D. R. Burton, J. Boyd, A. D. Brampton, S. B. Easterbrook-Smith, E. J. Emanuel, J. Novotny, T. W. Rademacher, M. R. van Schravendijk, M. J. E. Sternberg, and R. A. Dwek, Nature, 288, 338 (1980).

4) N. C. Hughes-Jones and B. Gardner, Immunology, 34, 459 (1978).

5) M. Cohn and J. Townsend, Nature, 173, 1090 (1954).

6) B. R. McGarvey, J. Phys. Chem., 61, 1232 (1957).

7) Y. Arata, A. Shimizu, and M. Matsuo, J. Am. Chem. Soc., 100, 3230 (1978).

8) Y. Arata and A. Shimizu, Biochemistry, 18, 2513 (1979).

9) A. Shimizu, M. Honzawa, Y. Yamamura, and Y. Arata, Biochemistry, 19, 2784 (1980).

10) Y. Arata, M. Honzawa, and A. Shimizu, Biochemistry, 19, 5130 (1980).

11) Modulation width, microwave power, and the position of capillary tube in a cavity were kept constant in all measurements. Mn ESR signal intensity was normalized by the intensity of a reference DPPH (1,1-diphenyl-2-picrylhydrazyl) signal in order to correct the Q value of the cavity. The average of three measurements was used as the observed value.

12) C. Tanford and J. D. Hauenstein, J. Am. Chem. Soc., 78, 5287 (1956).

13) T. Azuma, T. Hirai, K. Hamaguchi, and S. Migita, J. Biochem., 67, 801 (1970).

14) J. K. Weltman and M. Sela, Biochem. Biophys. Acta., 93, 553 (1964).

15) H. J. Gould, T. J. Gill, III, and P. Doty, J. Biol. Chem., 239, 2842 (1964).

(Received December 11, 1981)