^{1}H NMR Study of Dissociation and Re-association of Apoferritin and Ferritin

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Synopsis. The processes of dissociation and re-association of apoferritin and the protein part of ferritin were investigated by ^{1}H NMR. It was suggested that the re-association of ferritin occurs more easily than that of apoferritin.

Ferritin is an iron storage protein consisting of 24 subunits around the iron core which is the polymerized iron (the composition is $(FeOOH)_8(FeOOPO_3H_2)$) and contains 0—4000 iron atoms.[1] Apoferritin, the protein part of ferritin (M.W. 445000), can be dissociated to subunits by sodium dodecyl sulfate,[2] 7 M (1 M=1 mol dm^{-3}) guanidinium chloride[3] and 67% (v/v) acetic acid.[4] It has been shown that the dissociation of apoferritin is reversible; However a significant degree of hysteresis is known to exist in the re-association process.[5] In a previous paper,[6] we have applied the technique of nuclear magnetic resonance (NMR) to the study of ferritin to discuss the state and role of the phosphate group on the surface of the iron core. In the present experiment, it will be shown that the processes of dissociation and re-association of the protein assembly in ferritin and apoferritin can be followed by ^{1}H NMR. The NMR signal from the protein part can be observed only after the protein part dissociates into subunits (M.W. 18500). Of special advantage of NMR over other methods is the fact that, in the case of ferritin, the iron core influences only little the NMR signal of the protein. The process of dissolution of the iron core to iron(III) ion will also be discussed using the linewidth of the peak of solvent water. On the basis of these results, we will discuss a possible role of the iron core in the processes of dissociation and re-association of ferritin.

Horse spleen ferritin (Nutritional Biochemicals Corporation, twice recrystallized, Cd free) was dialyzed against dilute EDTA solution. All reagents used are of reagent grade. The concentration of iron was determined by using atomic absorption[6] and that of protein by Lowry method.[7] ^{1}H NMR spectra were recorded using a JEOL PS-100 spectrometer operating at 100 MHz.

The pH of sample solution was changed in either of the following two ways: For the study of dissolution of the iron core to iron(III) ion, a small amount of ferritin solution (0.35 M, as iron) was added to a glycine–HCl buffer solution with pH pre-adjusted, and the linewidth of the NMR signal of water in the solution was measured. Before and after the above treatment, no precipitation occurred and very little change in pH was observed. 2) For the study of the dissociation of protein part for apoferritin and ferritin, the pH of sample solutions was changed by dialyzing for 24 h against a 10 mM glycine–HCl buffer. In the experiment of re-association, the solution, which had been dialyzed for 24 h against a 10 mM glycine–HCl buffer at pH 1.8, was further dialyzed for 24 h against the same buffer at higher pH.

Dissociation of the Protein Assembly in Ferritin and Apoferritin. The NMR signal of the protein part becomes observable when the pH of solutions of ferritin or apoferritin decreases below 3.5, suggesting that the protein part dissociates into subunits. The aliphatic region of the NMR spectrum of the protein part is shown in Fig. 1A. The signal indicated by an arrow is presumably due to the methyl groups of leucine, isoleucine, and valine. The height of the peak reaches maximum below pH 1.8; the extent of dissociation of the protein part was determined by measuring the height of these peaks. The dissociation curves for apoferritin and ferritin thus obtained are shown in Fig. 1B and Fig. 2, respectively; no significant difference was observed between ferritin and apoferritin.

The Process of Dissolution of the Iron Core to Iron(III) Ion. The red color of the ferritin solution fades when the pH decreases below 2.0, where the linewidth of H_2O signal greatly increases. This is most likely because the iron core dissolves and the amount of aqueous iron(III) ion increases at low pH, resulting in a large increase in the interaction of water molecule with the paramagnetic iron(III) ion. This suggests that the process of the dissolution of the iron core to iron(III) ion may

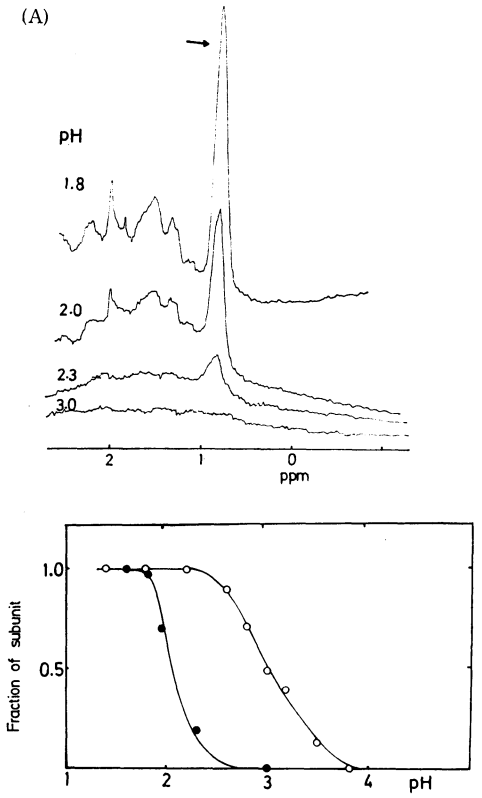

Fig. 1. (A) The aliphatic region of 100 MHz NMR spectra of apoferritin at low pH. The protein concentration was 12 mg/ml. (B) Dissociation and re-association of apoferritin at low pH.
●: Dissociation, ○: re-association.

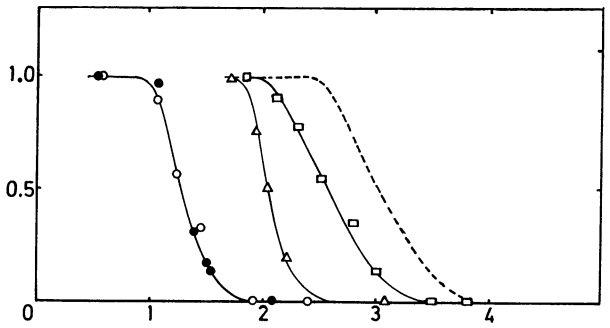

Fig. 2. Dissociation and re-association of ferritin at low pH.
○: Dissolution of iron core (NMR), ●: ($OD_{410\ nm}$), △: dissociation, □: re-association.

be followed quantitatively by observing the linewidth of the water signal. In the present experiment, the concentration of the ferritin solutions was 5 mM (as iron); at this concentration the solution is clear and there is no precipitation nor aggregation observed with the dissolution of the iron core. The results shown in Fig. 2 indicate that the iron core begins to dissolve at pH 1.8 and dissolves completely at pH 1.0. This process was also followed by the measurement of $OD_{410\ nm}$ and the result is in good agreement with that obtained by the NMR measurement. See Fig. 2. The results in Fig. 2 suggest that the protein part of ferritin begins to dissociate at pH 3.5 and dissociates completely at pH 1.8, but the iron core itself remains intact above pH 1.8. It is of interest that in a narrow range around pH 1.8 the protein part dissociates completely with the intact iron core.

Re-association of Subunits of Ferritin and Apoferritin. With an increase in pH of the solutions of ferritin or apoferritin where the protein part has been dissociated at low pH, the NMR signals of the protein part becomes undetectable, suggesting that the subunits re-associate. The fraction of re-association was determined from the peak height as in the case of dissociation. The re-association curve of apoferritin is shown in Fig. 1B. In this experiment, 0.01 M glycine–HCl buffer was used in dialysis at pH 1.8 to dissociate and at higher pH to re-associate the protein part. A similar experiment was carried out using ferritin. The re-association curve for ferritin is also included in Fig. 2. The broken line is the re-association curve of apoferritin. The curve of re-association for ferritin, which has already been dissociated at pH 1.8, deviates greatly from that for apoferritin. This suggests that re-association of the protein part occurs more easily in the case of ferritin than in the case of apoferritin.

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