The binding analysis of commercial horse spleen ferritin to beads (PLB) immobilized with PL and the binding of its apoferritin form to PLB and CasB. A) PLB was prepared using CNBr-activated Sepharose 4B with PL (Wako Pure Chemical Corporation, Osaka, Japan) according to the method described previously [9]. Twenty-five micrograms of commercial horse spleen ferritin was added to 1 ml PBS containing PLB or CB (net volume of beads per sample: 20 μl), and the mixture was rotated at 4°C for 30 min. The mixture was centrifuged at 16,000 x g for 5 min at 4°C, and then the pelleted beads were suspended with 1 ml of PBS. The mixture was again centrifuged under the same centrifugation conditions and the pelleted beads were washed three times with 1 ml of PBS under the same centrifugation conditions as described in Figure 1.

The supernatant (S) was collected after the first centrifugation before washing the beads. B) Forty micrograms of commercial horse spleen apoferritin was added to 1 ml PBS (net volume of beads per sample: 20 μl) containing PLB or CasB, and the mixture was rotated at 4°C for 30 min. The mixture was centrifuged under the same centrifugation conditions as described above, and the pelleted beads obtained were washed using the same centrifugation conditions. The supernatant (S) was collected after the first centrifugation before washing the beads. The proteins obtained by A and B were subjected to SDS-PAGE together with each S sample, followed by staining with Coomassie Brilliant Blue R250. CB was treated by the same methods. Horse spleen ferritin (HFt) and apoferritin (apo-HFt) was separately applied to the gel (each 2 μg/lane). Arrows indicate holo- (A) or apo-ferritin (B), but CasB did not show the binding with apo-HFt. M represents marker proteins.