

2-19. Purification and Characterization of
Haemolymph Ferritin from the Cricket,
Gryllus bimaculatus

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The isolation and purification of ferritin from the cricket, *Gryllus bimaculatus* haemolymph were accomplished by anion exchange column chromatography using HiTrap Q column (1.6 x 4 cm, Amersham Pharmacia Co.), 70°C heat and acid treatment, and gel filtration column chromatography using G4000SW column (0.75 x 60cm, TOSOH Co.) by FPLC system. Ferritin was found to have one major subunit (32 kDa) and four minor subunits (30, 28, 27 and 25 kDa) by 2D-PAGE, and represented homogeneous and regular 8 nm core by EF-TEM. Amino acid composition analysis of ferritin showed that Met, Leu and Lys were in high concentration, while Cys, Tyr and Trp in low concentration. *G. bimaculatus* haemolymph ferritin is considered to be one of methionine-rich proteins. The N-terminal amino acid sequences of 32 kDa subunit was analyzed using ESI-MS by internal digestion, because N-terminus of the subunit was blocked. Three partial internal amino acid sequences were obtained; VLELDPESQPR, GMLAMSELESLGK and ADGLQVFLL.