Title: Evidence for a monomer-dimer equilibrium in native human Factor H

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Abstract

Factor H (FH) plays a central role in the regulation of complement activation via the alternative pathway. It is composed of 20 short complement regulator (SCR) domains. Previously, we had demonstrated that Factor H is dimeric in solution by X-ray and neutron scattering (Perkins et al. (1991) *Biochemistry*, **30**, 2847-2857), while the follow-up study demonstrated that Factor H is monomeric (Aslam & Perkins (2001) *J. Mol. Biol.* **309**, 1117-1138). Recently the SCR-6/8 and SCR-16/20 fragments of Factor H have each revealed monomer-dimer self-association with K_D values of 40 μM and 16 μM respectively. We have reinvestigated the discrepancy in the earlier studies by the use of c(s) size-distribution analyses of Factor H in sedimentation velocity experiments on the analytical ultracentrifuge. Between 0.1 mg/ml to 0.6 mg/ml of Factor H in either phosphate buffer saline or Hepes buffer, two peaks were observed in the c(s) analyses between 25k r.p.m. and 50k r.p.m. rotor speeds. The major peak at 5.7 ± 0.1 S corresponds to a molecular weight at 142 ± 2 kDa, while the second peak at 9.2 ± 0.5 S corresponds to a molecular weight at 285 ± 24 kDa. The relative intensity of the second peak increased with concentration. We conclude that Factor H exists in a monomer-dimer equilibrium in solution. A tentative explanation of the outcomes of the 1991 and 2001 studies is that these were fortuitously performed in concentration ranges that corresponded to predominantly dimeric and monomeric Factor H. Further data collection is in progress and will be presented alongside a reanalysis of our 2001 data. From the results so far obtained, we conclude that Factor H will be predominantly monomeric in plasma in its physiological concentration range between 0.2 mg/ml to 0.8 mg/ml.