HEME INHIBITS THE CLASSICAL COMPLEMENT PATHWAY BY BINDING TO C1Q

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Supplemental Experimental Procedures

Interaction of native and heme exposed C1q to IgG1, monomeric and pentameric CRP,

The binding of C1q to its targets was studied by surface plasmon resonance-based technology BIAcore2000, (Biacore GE Healthcare, Uppsala, Sweden). IgG1 or CRP were always diluted in 5 mM maleate pH 5 and pH 3.8, respectively to final concentration 100 μg/ml and coupled to a CM5 sensor chips (Biacore) by using a standard amine coupling protocol, provided by the manufacturer (Biacore). CM5 sensor chip contains dens layer of semi-solid carboximethylated dextran that offer immobilized proteins to behave as in solution. IgG1 was coupled with efficiency of about 15000 resonance units (RU). CRP was coupled with efficiency of about 6800 RU. Monomeric CRP was obtained by a brief exposure (30 sec) of the sensor surface to 3.5M guanidine.HCl. In this case the baseline response decreased from about 6800 to about 1900RU, correlating with a dissociation of the pentameric structure. This preparation of CRP will be further referred to as monomeric CRP (mCRP). CRP that was not treated with guanidine.HCl is referred as pentameric (pCRP). The running buffer throughout the study was PBS pH 7.4 (DPBS Gibco, 0.22 μm filtered and degassed) and the regeneration buffer was 1.25 M guanidineHCl. At this concentration of guanidine.HCl the pentameric structure of CRP was maintained on the sensor chip, since the baseline remained stable. The regeneration buffer was selected on the basis of its efficacy to regenerate the chip surface, removing residually bound C1q. The interaction of C1q with IgG1, mCRP and pCRP were measured by series of injections of different concentrations of C1q (5, 2.5, 1.25, 0.625, 0.313, 0.156 nM). Further 100 nM C1q was treated with different concentrations of DMSO-diluted hemin (Fe(III)PP), Zn(II)PP, Mn(III)PP, Ni(II)PP, Sn(IV)MP, Cr(III)PP, Co(III)PP, HPIX or DMSO alone (0 - 30 μM). MgPP was dissolved in water and processed as the other porphyrines. All above mentioned experiments were performed at 25°C.

Evaluation of binding thermodynamics

In brief, the kinetic rate constants obtained at different temperatures were used to build Arrhenius plots (reciprocal value of the temperature in Kelvin versus natural logarithm of the rate constants). The slopes of the plots were calculated by linear regression analysis using GraphPad Prism 5 software (Graph Pad, San Diego, CA) and subsequently substituted in the Arrhenius equation:

$E_a = -slope \times R$,

where the "*slope*" = $\partial \ln k_{a/d} / \partial(1/T)$,

where E_a is the activation energy and R is the universal gas constant ($R = 8.3$ J mol^{-1} K^{-1}). The enthalpy, entropy and Gibbs free energy changes characterizing the association or dissociation phases were calculated using the equations:

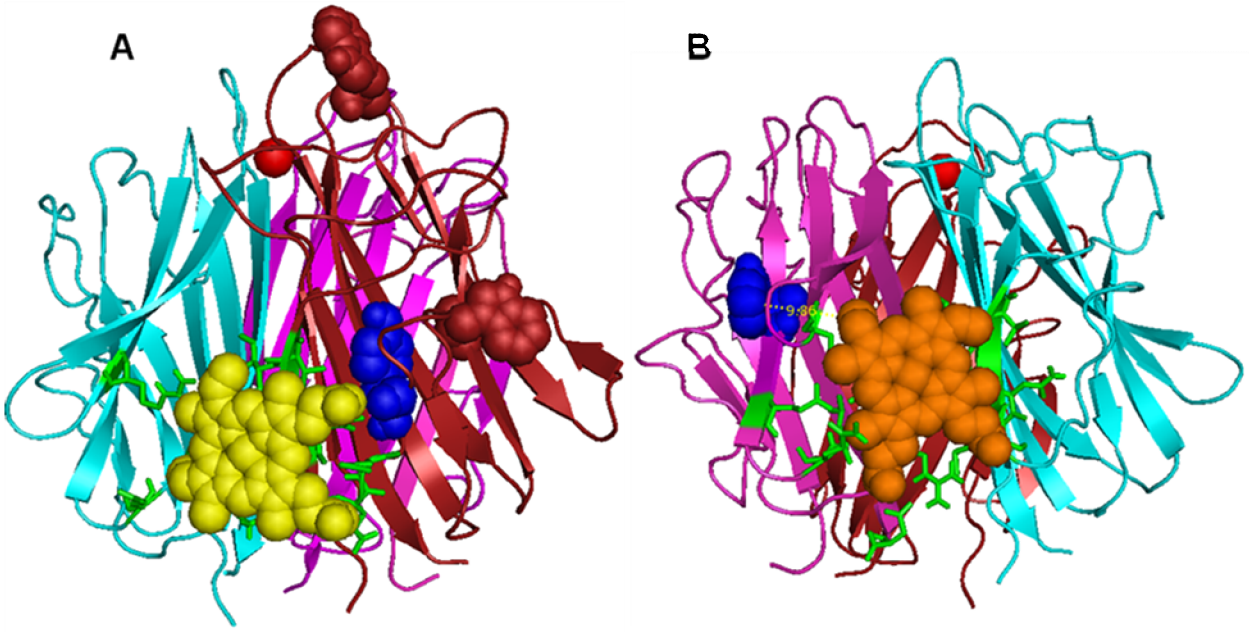
$\Delta H = E_a - RT$

$\ln(k_{a/d}/T) = -\Delta H/RT + \Delta S/R + \ln(k'/h)$

$\Delta G = \Delta H - T\Delta S$,

where T is the temperature in the Kelvin scale, k' is the Boltzman constant and h is the Planck's constant.

Supplemental Figure



Supplemental Figure 1. Description of the most probable heme-binding sites A) A-chain heme binding site consists of ValA^{114}, IleA^{115}, AsnA^{117}, GluA^{119}, GluA^{120}, AsnA^{124}, ArgB^{159}, LysB^{188}, GluB^{190}, depicted in green in a stick representation. AsnA^{117} makes a contact with TyrA^{122} (blue, spheres representation), which is at about 3Å from the heme molecule. Two tryptophan residues are present in A chain (brick red, spheres representation). TrpA^{194} is at about 13Å distance from the heme molecule. Heme molecule is depicted in yellow in a sphere representation. B) The C-chain heme binding site consists of ValB^{118}, IleB^{119}, ThrB^{120}, AsnB^{121}, MetB^{122}, AspC^{156}, ArgC^{182}, LeuC^{183}, GlnC^{184}, GluC^{187} and AspC^{217}, depicted in green in a stick representation. In the globular fragments of the B and C-chain of C1q there is only one Trp residue – TrpC^{190} (blue, spheres representation) and it is at less then 10 Å from the heme molecule, close to ArgC^{182} and GluC^{187} which make direct contact with the heme. Heme molecule is depicted in orange in a surface representation. In the two figures the A-chain is in brick red, the B-chain is cyan and the C-chain is in magenta. The Ca2+ ion is a red sphere.