

In Archaea and Bacteria, surface layer (S-layer) proteins form the cell envelope and are involved in cell protection. In the present study, a putative S-layer protein was purified from the crude extract of *Pyrococcus horikoshii* using affinity chromatography. The S-layer gene was cloned and expressed in *Escherichia coli*. Isothermal titration calorimetry analyses showed that the S-layer protein bound *N*-acetylglucosamine and induced agglutination of the gram-positive bacterium *Micrococcus lysodeikticus*. The protein comprised a 21-mer structure, with a molecular mass of 1,340 kDa, as determined using small-angle X-ray scattering. This protein showed high thermal stability, with a midpoint of thermal denaturation of 79 °C in dynamic light scattering experiments. This is the first description of the carbohydrate-binding archaeal S-layer protein and its characteristics.