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Immobilization of bacteriophage Qβ on metal-derivatized surfaces via polyvalent display of hexahistidine tags

Andrew K. Udit *, Steven Brown, Michael M. Baksh, M.G. Finn *

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

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ABSTRACT

Metal-binding peptide motifs are widely used for protein purification, catalysis, and metal-mediated self assembly in the construction of novel materials and multivalent light harvesting complexes. Herein we describe hexahistidine sequences incorporated into the virus-like particle derived from bacteriophage Qβ via co-expression of the wild-type (WT) and hexahistidine-modified coat proteins in *Escherichia coli*. The resulting polyvalent display of approximately 37 hexahistidine moieties per virion gave rise to altered properties of Zeta potential and hydrodynamic radius, but no observed change in stability compared to WT. While the resulting display density did not permit hexahistidine chains to cooperate in the coordination of heme, the multiple tags did impart a strong affinity for immobilized metal ions. A dissociation constant for binding to Ni–NTA of approximately 10 nM was measured by SPR under non-competitive, physiological conditions. Affinity chromatography over immobilized metal columns was used to purify the particles from both crude cell lysates and after chemical derivatization. These results illustrate the potential of metal-NTA surfaces for the self-assembled presentation of multi-functionalized particles to interrogate systems ranging from small molecule binding to whole cell interactions.

1. Introduction

The utility of polyhistidine and other metal-binding motifs incorporated into protein structures has expanded beyond protein purification to include applications in water purification [1], handles for protein–protein interactions to yield novel materials and structures [2], and surface immobilization. The latter use has received particular interest for applications in nanopatterning [3], biosensors [4] and surface-bound catalysts [5]. Perhaps most compelling are potential uses of polyvalent protein scaffolds such as virus particles where several groups have already demonstrated their utility in battery design [6], metal nucleation and deposition [7], and MRI contrast agents [8].

Previous work in our laboratory with the hepatitis B virus capsid protein has yielded a virus-like species that polyvalently displays a hexahistidine tag at each of its 240 subunits, designated HBV-$(His_6)_{240}$ [9]. Added heme cofactor was bound tightly by these particles via bis-histidine coordination, with one heme incorporated for every three capsid proteins. The virus-bound hemes displayed chemical reactivity, electrochemical response, and absorption spectra characteristic of b-type cytochromes. Interestingly, the particles showed no affinity for Ni(II) during immobilized metal affinity chromatography (IMAC), suggesting that the peptides were buried within the capsid superstructure. This precluded use of the hexahistidine tags for applications dependent on surface immobilization. To extend these studies, we sought to exploit the polyvalent nature and straightforward genetic manipulation of a robust self-assembling capsid protein derived from a different structure. Here we describe the use of bacteriophage Qβ, an icosahedral RNA virus comprised of 180 copies of a 14.1 kDa, 132 amino acid coat protein [10] that is readily expressed and purified from *Escherichia coli* [11]. Prior studies have shown that the coat protein can tolerate some genetic insertions [12], and we have reported several mutants bearing modest sequence alterations, including the incorporation of unnatural amino acids [13–17].

2. Results and discussion

While some point mutations of the wild-type (WT) Qβ sequence are well tolerated by the system, allowing for high expression yields of protein and spontaneous formation of particles in large amounts, others are known to dramatically affect capsid stability [18,19]. Our initial attempts at more dramatic changes to provide metal binding motifs were largely unsuccessful. For example, various C-terminal fusions gave copious quantities of the coat protein but no isolable intact virions, suggesting that protein folding and/

* Corresponding authors. Present address: Occidental college, Department of Chemistry, 1600 Campus Road, Los Angeles, CA 90041, USA. Tel.: +1 323 259 1471 (A.K. Udit).
E-mail addresses: udit@oxy.edu (A.K. Udit), sdbrown@scripps.edu (S. Brown), mmbaksh@scripps.edu (M.M. Baksh), mgfinn@scripps.edu (M.G. Finn).

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or particle assembly was compromised by the added residues. Similar results were obtained for coat proteins within which residues clustered at subunit boundaries were changed to histidine. Thus, to obtain capsids displaying potential metal-binding motifs we turned to a co-expression method based on the work of Pumpens et al. [12], in which stable mixed particles incorporating both WT and C-terminal fusion coat proteins are generated. The fusion component can contain large appended domains or full-length proteins (to be described elsewhere) [20].

Qβ coat protein co-expression was achieved using two plasmids in *E. coli*. The peptide sequence GSGSGHHHHHH was appended to the C-terminus of the WT coat protein in the spectinomycin resistant pCDF-1b expression vector; the GSGSG spacer was included to allow the hexahistidine moiety greater flexibility for interaction with metal ions in solution. Simultaneous transformation of *E. coli* with the pCDF-1b construct and the WT coat protein in the carbenicillin resistant pET-11d expression vector yielded bacteria possessing both IPTG-inducible plasmids when grown in spectinomycin-carbenicillin media. Coat protein expression and particle purification following our standard protocol produced intact virions in good yield (20 mg virus per liter of culture) as observed by size-exclusion fast protein liquid chromatography (FPLC). Fig. 1a displays the resulting FPLC elution profile for the hexahistidine-containing Qβ particle which is indistinguishable from that of WT. Analysis of the particles by denaturing SDS–PAGE (see Supplementary material, Fig. S1) confirmed the presence of both WT and hexahistidine-bearing capsid proteins at 14.1 and 15.3 kDa, respectively, in a ratio of 3.9:1 as measured by densitometry. MALDI-TOF mass spectrometry (Fig. 1b) also showed two peaks, corresponding to WT (obs. 14220 Da, calc. 14238 Da) and hexahistidine (obs. 15399 Da, calc. 15406 Da) coat protein components, in the same 3.9:1 ratio. Thus, each composite virion possessed an average of approximately 37 copies of the hexahistidine peptide per 180-subunit particle, and is therefore designated as Qβ-$(His_6)_{37}$. Repeated independent preparations under identical expression conditions showed this value to be quite reproducible, in accord with results for other co-expressed constructs [21].

As might be expected, the multiple hexahistidine tags incorporated onto the Qβ scaffold were found to alter the physical properties of the particle. For example, dynamic light scattering showed the WT particle to have a hydrodynamic radius of 14.7 nm and a narrow polydispersity of 9.9%, both values consistent with the known capsid structure. In contrast, the measured average radius of Qβ-$(His_6)_{37}$ was 19.5 nm with a polydispersity of 32.6%; larger size and polydispersity are both expected of a particle incorporating variable amounts of the 11-residue C-terminal extension by a spontaneous self-assembly process. Light scattering measurements performed in the presence of 0–100 μM Ni(II) (maximum molar ratio of 50:1 for Ni(II) to hexahistidine subunit) did not yield any significant changes in the observed hydrodynamic radius of Qβ-$(His_6)_{37}$. Thus, polyvalent presentation of the metal-binding motif does not appear to induce particle aggregation, either from inter-particle hexahistidine-mediated metal chelation or metal-induced flocculation. Interestingly, the Zeta potential of WT and Qβ-$(His_6)_{37}$ were found to be 0.6 ± 0.5 and −2.9 ± 0.4 mV, respectively. That the addition of cationic hexahistidine motifs imparts a more negative surface potential to the virion may indicate that the surface ion adsorption profiles of the two particles differ, with the relatively cationic Qβ-$(His_6)_{37}$ attracting more anions to yield particles with negative surface potentials. This would be expected to enhance the difference in hydrodynamic radius for the composite *vs.* the wild-type Qβ capsid, and may contribute to the larger-than-expected change of approximately 33% in the measured radius of the particle.

In contrast to our studies with HBV-$(His_6)_{240}$, the Qβ-$(His_6)_{37}$ particles showed strong interactions during IMAC (Fig. S2). While the WT particles had no propensity to bind columns charged with Ni(II), the Qβ-$(His_6)_{37}$ virions were trapped efficiently on the resin. Elution was initiated at an imidazole concentration of 170 mM, with peak elution occurring at 264 mM, indicating strong binding to the immobilized metal centers. Disassembled Qβ-$(His_6)_{37}$ subunits, obtained by treatment of particles with 4 M urea and 2% w/v SDS with 5 mM DTT at 95 °C for 30 min, exhibited peak elution at 140 mM imidazole (data not shown). While the particle therefore apparently interacts with the column more strongly, polyvalent (simultaneous multi-point) binding is not necessarily indicated. For example, a cooperative binding effect was recently reported in an analogous experiment with green fluorescent protein (GFP) bearing two sequential hexahistidine tags, compared to two tags individually isolated at the N- and C-termini. In this study the isolated and sequentially tagged constructs eluted from Ni–NTA agarose with imidazole concentrations of 0.1 and 0.6–0.8 M imidazole, respectively [22], a significantly greater increase in the required strength of the elution buffer than we observe. Interestingly, the nature of the immobilized cation did not affect the elution profile of Qβ-$(His_6)_{37}$: HiTrap IMAC columns charged with any of Zn(II), Ni(II), Co(II), or Cu(II), gave rise to very similar elution profiles for Qβ-$(His_6)_{37}$, with peak elution occurring near 250 mM imidazole (Table S1). This insensitivity is unusual as other reports have generally described some metal-dependent retention of affinity-tagged proteins during IMAC [23–25].

The strong affinity of Qβ-$(His_6)_{37}$ for metal-derivatized substrates was further explored with surface plasmon resonance (SPR). Commercially-available underivatized gold SPR plates were modified to display Ni(II)–NTA moieties. When exposed to solutions containing Qβ-$(His_6)_{37}$ (PBS buffer, pH 7.4, 27 °C) a 10-fold increase in signal intensity was observed relative to control surfaces bearing only 11-mercaptoundecylamine (MUAM) (Fig. 2a). The resulting equilibrium binding measurements (Fig. 2b) were fitted to the classical Langmuir equation (monovalent binding) to give a dissociation constant of 1.15 ± 0.37 × 10^{-8} M, an order of magnitude greater affinity than is typically observed for hexahistidine-

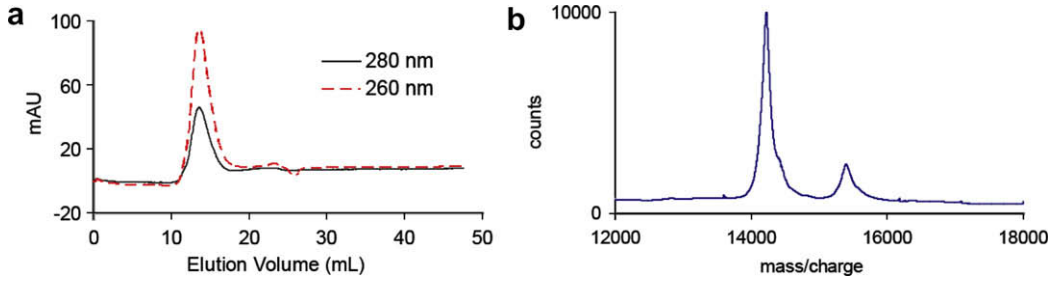

Fig. 1. (a) Size-exclusion FPLC of Qβ-$(His_6)_{37}$ in 0.1 M KPi pH 7 buffer after purification by standard ultracentrifugation protocols; the elution volume and A260/A280 ratio are identical to those of the wild-type capsid. (b) MALDI-TOF mass spectrum of Qβ-$(His_6)_{37}$ showing both WT (14220 m.u.) and hexahistidine modified (15399 m.u.) coat proteins.