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Cell-free production of trimeric influenza hemagglutinin head domain proteins as vaccine antigens

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Abstract

In order to effectively combat pandemic influenza threats, there is a need for more rapid and robust vaccine production methods. In this paper, we demonstrate *E. coli*-based cell-free protein synthesis (CFPS) as a method to rapidly produce domains from the protein hemagglutinin (HA), which is present on the surface of the influenza virus. The portion of the HA coding sequence for the ‘head’ domain from the 2009 pandemic H1N1 strain was first optimized for *E. coli* expression. The protein domain was then produced in CFPS reactions and purified in soluble form first as a monomer and then as a trimer by a C-terminal addition of the T4 bacteriophage foldon domain. Production of soluble trimeric HA head domain was enhanced by introducing stabilizing amino acid mutations to the construct in order to avoid aggregation. Trimerization was verified using size exclusion HPLC, and the stabilized HA head domain trimer was more effectively recognized by antibodies from pandemic H1N1 influenza vaccine recipients than was the monomer and also bound to sialic acids more strongly, indicating that the trimers are correctly formed and could be potentially effective as vaccines.

Keywords

Hemagglutinin; influenza; trimer; cell-free protein synthesis

Introduction

In the United States alone, infections from the influenza virus lead to 20,000-40,000 deaths and 200,000 hospitalizations each year with worldwide figures approximately an order of magnitude higher (Thompson et al., 2003; Thompson et al., 2004). While vaccines are commonly available, circulating influenza viruses typically undergo several point mutations from year to year (referred to as antigenic drift) so that yearly vaccinations are required. Furthermore, the recombination of multiple influenza strains, including strains from

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different hosts (e.g. avian and swine), can create new viral subtypes (antigenic shift) and lead to pandemics like the 1918 influenza pandemic which killed tens of millions (Johnson and Mueller, 2002) and the 2009 'swine flu' which led to over 15,000 deaths and a level 6 (highest level) pandemic alert (Girard et al., 2010). Current commercial influenza vaccines are produced in embryonated chicken eggs. The process is lengthy and laborious, requiring a minimum of six months preparation, making it largely ineffective for combating potential pandemics. The process also yields only approximately one dose per egg, putting great strains on production during years when larger amounts of vaccine are required (Biesova et al., 2009). Furthermore, egg-based vaccines are susceptible to microbial contamination which has led to shortages in the past (Fink, 2004). Cell culture-based vaccine production is a possible alternative and has shown efficacy in Phase III clinical trials (Barrett et al., 2011). However, the process would still likely only improve production times by about a month. Thus, a more effective vaccine that can be produced in a faster, more scalable manner would be very useful in avoiding influenza infections and preventing pandemics.

Influenza viruses are classified by their two surface proteins, hemagglutinin (HA) and neuraminidase (NA). The more abundant HA protein is the primary protective antigen and is a typical target when trying to devise more effective vaccines (Stevens and Donis, 2007). HA is an integral membrane glycoprotein that exists as a homotrimer of three approximately 75 kDa protein monomers (Wilson et al., 1981). The HA ectodomains consist of a head domain responsible for binding to sialic acid residues located on cell surface receptors and a stem domain responsible for fusion to endocytic vesicles inside host cells.

The majority of protective antibodies generated from the immune response to current vaccines bind to the HA head domain, and significant efforts have been made to produce vaccines comprising this more immunoprotective domain. Because the head domain consists of mostly compact, globular β -sheets, this domain folds much more readily than the loose, extended coils that comprise the stem domain. Several groups have successfully prepared HA head domain vaccines either alone or with adjuvants by refolding *E. coli*-produced HA head antigen. Several of these vaccines have been shown to be effective in animal models (Song et al., 2008; Wei et al., 2008; Song et al., 2009; Aguilar-Yanez et al., 2010; Alvarez et al., 2010; Khurana et al., 2010; DuBois et al., 2011). However, to our knowledge, no scalable process has emerged for producing the native trimeric conformation.

In this paper, we demonstrate the production of HA head domain from the 2009 pandemic H1N1 strain in either monomeric or trimeric form without the need for refolding by using *E. coli*-based cell-free protein synthesis (CFPS) production methods. Recent advances have shown *E. coli*-based CFPS to be a robust and scalable platform for producing a wide variety of proteins (Voloshin and Swartz, 2005; Swartz, 2006; Zawada et al., 2011), including heterologous proteins that contain disulfide bonds (Kim and Swartz, 2004; Yin and Swartz, 2004; Goerke and Swartz, 2009). Thus, production of the HA head domain is another example of the utility of this method for recombinant protein production. The properties of the HA head domain monomer and trimer were then compared using several analytical assays. Formation of the HA head domain trimer was confirmed using size exclusion chromatography. The trimer, as compared to the monomer, was further shown to more effectively bind both antibodies from vaccine recipients of the strain specific 2009 pandemic

H1N1 virus as well as sialic acid residues on the surface of the glycoprotein, fetuin. Therefore, the trimer displays both structural and functional differences compared to the monomer. These CFPS-produced HA head domains could provide a valuable antigen for use in combating influenza infections. The production methods provided by CFPS would allow rapid production of the HA proteins as potent vaccines to stop pandemics and to respond to annual threats. Additionally, a library of such reagents could be used to screen patient-derived antibodies to quickly identify the best vaccine to combat emerging strains, further increasing the response speed (Alvarez et al., 2010).

Materials and Methods

Construction of Vectors for CFPS Reactions

Constructs for CFPS expression of the HA head domain were cloned into the pY71 vector using NdeI and AatII restriction sites. pY71 is a reduced size plasmid (2.45 kb) that utilizes the T7 promoter and contains a pUC19 origin of replication and a kanamycin resistance element (Kuchenreuther et al., 2009). Based on standard H3 numbering, residues K63-S270 of the HA ectodomain from the influenza strain A/California/07/2009(H1N1) (GenBank accession No. ACQ55359.1) were used to produce the HA head domain (Wilson et al., 1981; Stevens et al., 2004). To maximize translation rates in the CFPS system, the sequence was first modified for *E. coli* Class II expression (codon bias during exponential growth phase) using DNAWorks and assembled using overlapping primer PCR (Hoover and Lubkowski, 2002). The 5' end of the construct was further modified using M-fold to reduce formation of secondary structure near the ribosome binding site (Zuker, 2003). The first 21 nucleotides of the coding sequence were as follows: ATGAAATGCAACATCGCTGGT. The following sequence was used to add a foldon trimerization sequence and *Strep*-tag II purification tag at the C-terminus of the construct, preceded by a cleavable TEV protease site: enlyfqsgsgyipeaprdgqayvrkdgewvllstflg **GGGGSWSHPQFEK** Lower case represents the TEV protease target sequence, CAPS represent the foldon sequence, and **BOLD CAPS** represent the *Strep*-tag II sequence. The nucleotide sequence for the foldon domain was also optimized for *E. coli* Class II expression.

Cell-Free Protein Synthesis

CFPS was conducted using the PANox-SP (PEP, amino acids, nicotinamide adenine dinucleotide (NAD), oxalic acid, spermidine, and putrescine) cell-free system as described previously (Jewett and Swartz, 2004) with several modifications. The standard PANox-SP CFPS reaction mixture includes: 1.2 mM ATP, 0.85 mM each of GTP, UTP, and CTP, 33 mM phosphoenol pyruvate (Roche Molecular Biochemicals, Indianapolis, IN), 170 mM potassium glutamate, 10 mM ammonium glutamate, 16 mM magnesium glutamate, 1.5 mM spermidine, 1.0 mM putrescine, 0.17 mg/mL folinic acid, 13.3 µg/mL plasmid, approximately 100–300 µg/mL T7 RNA polymerase, 2 mM of each of the 20 unlabeled amino acids, 0.33 mM NAD, 0.26 mM Coenzyme A (CoA), 2.7 mM potassium oxalate, and 0.28 volumes of *E. coli* KGK10 S30 extract (Knapp et al., 2007; Goerke and Swartz, 2008). For CFPS reactions containing the GroEL/S chaperonin complex, the *E. coli* BL21(DE3)Star strain (Invitrogen, Carlsbad, CA) harboring the pGroESL plasmid (Goloubinoff et al., 1989) was used in place of the KGK10 strain. GroEL/S production was

induced with the addition of 1 mM IPTG at an OD₆₀₀ of 0.6 during extract preparation. All reagents were obtained from Sigma–Aldrich (St. Louis, MO) unless otherwise noted.

T7 RNAP used in reactions was prepared using a modified version of a previously described protocol (Li et al., 1999). Briefly, 1 L cultures of BL21(DE3) pLysS cells supplemented with 100 µg/mL ampicillin and harboring the pAR1219 plasmid were grown in LB media at 37 °C and induced with 1 mM IPTG at 0.6 OD₆₀₀. After 4 h, cells were harvested by centrifugation at 6,000 × g for 30 min. Pellets were resuspended in 10 mM potassium phosphate pH 7.6, 50 mM sodium chloride, 10 mM EDTA, 1 mM DTT, 10 mM β-Mercaptoethanol with one-half tablet of Roche Protease Inhibitor (Roche Molecular Biochemicals). Cells were lysed with a single pass through an Avestin, Inc. (Ottawa, Canada) EmulsiFlex C-50 high-pressure homogenizer at 20,000 psig. Lysate was centrifuged sequentially at 20,000 × g for 30 min and 150,000 × g for 1 h and then dialyzed in 40 mM potassium phosphate pH 7.6, 50 mM sodium chloride, 1 mM EDTA, 1 mM DTT, and 20% sucrose. The final sample was used in CFPS reactions at 200-fold dilutions.

Several modifications to PANOx SP CFPS were made to encourage disulfide bond formation. First, the cell extract was pretreated at room temperature for 30 min with 50 µM iodoacetamide (IAM). (For reactions utilizing the cell extract containing overexpressed GroEL/S chaperonin complex, 1 mM IAM was used.) Prior to template DNA addition, a glutathione buffer (4 mM oxidized glutathione and 1 mM reduced glutathione) was added to the cell-free reaction to stabilize the thiol/disulfide redox potential. Finally, DsbC, a periplasmic disulfide bond isomerase, was prepared as described previously (Goerke and Swartz, 2008) and added to a final concentration of 100 µg/mL.

CFPS reactions to produce the stem domain were conducted at 25 °C for 12 h. Small-scale CFPS reactions were carried out in 25 µL volumes in 1.5 mL microcentrifuge tubes. Preparative-scale reactions used 3 mL volumes with 1 mL per well in 6-well tissue culture plates (BD Falcon #3046, BD, Franklin Lakes, NJ). 8.4 µM L-[U-¹⁴C]-Leucine (PerkinElmer, Waltham, MA) was added to small-scale reactions and to 30 µL aliquots of preparative-scale reactions for measuring protein yields using a previously described trichloroacetic acid protocol (Calhoun and Swartz, 2005) and a Beckman LS3801 liquid scintillation counter (Beckman Coulter, Fullerton, CA). The soluble fraction of preparative-scale reactions was recovered by centrifugation at 21,000 × g for 15 min for further evaluation and purification.

Purification of HA Head Domain Proteins

Soluble CFPS products from 3 mL reactions were purified using the *Strep*-tag II/*Strep*-tactin system (IBA GmbH, Gottingen, Germany). The CFPS reaction product supernatants were dialyzed in Strep Load Buffer, SLB (100 mM Tris-HCl pH 8, 150 mM sodium chloride, 1 mM EDTA) and were concentrated with a second dialysis in SLB containing 20% sucrose. Further concentration from 1.5-2 mL to a final volume of 0.5-1 mL was performed using a 3 mL stirred-cell concentrator with a 5,000 kDa MWCO regenerated cellulose membrane (Millipore, Billerica, MA). The concentrated crude product solution was then applied to a 0.5 mL Strep-Tactin gravity flow column (IBA GmbH) and washed with 12 column volumes (CVs) of SLB. The loaded column was eluted with SLB containing 2.5 mM desthiobiotin

and 0.01% Tween-20, and 0.5-1 mL fractions were analyzed for protein content using SDS-PAGE gels. Pooled fractions were then dialyzed in 100 mM Tris-HCl pH 8, 150 mM sodium chloride, 0.01% Tween-20 and stored at -80°C .

Size Exclusion HPLC

Purified HA head domain proteins were tested on a Discovery Bio GFC 150 HPLC column, 15 cm $\times$ 4.6 mm inner diameter with 5 μm particles (Sigma-Aldrich). The running buffer was 10 mM Tris-HCl pH 7.5, 100 mM sodium chloride pumped at 0.2 mL/min. The injection volume was 25 μL . Protein absorbance was monitored in-line at 210 nm over a period of 35 min.

ELISA Analysis of HA Head Domain Proteins

To perform an enzyme-linked immunosorbent assay (ELISA) of the HA proteins, 70 μL of a commercial HA protein preparation and HA head domain proteins at 5 $\mu\text{g/mL}$ concentrations were coated on 96-well ELISA plates (Microton, flat-bottom, high binding; Greiner Bio One, Frickenhausen, Germany) and allowed to bind overnight at 4°C . Commercial HA consisted of amino acids 18-529 of the 2009 pandemic H1N1 strain (HA(TM)(A/California/07/2009(H1N1))) followed by a His₆ purification tag at the C-terminus (Immune Technology Corp., New York, NY). Plates were then washed twice with wash buffer (PBS buffer with 0.1% Tween-20) and blocked with PBS buffer with 3% bovine serum albumin and 0.02% NaN₃ (blocking buffer) at 37°C for 1 h. After washing four times with wash buffer, 70 μL dilutions of 1.9 $\mu\text{g/mL}$ pooled plasmablast-derived polyclonal antibodies (PPAb, see below) from influenza vaccine recipients were then added to the plates and incubated at 37°C for 1 h. Plates were again washed six times with wash buffer before adding peroxidaseconjugated goat anti-human IgG γ antibody (KPL, Gaithersburg, MD) at a 1:4000 dilution in blocking buffer and incubating at 37°C for 1 h. Plates were again washed six times before developing with 70 μL of tetramethylbenzidine substrate (KPL) and quenching with 30 μL of 2% H₂SO₄. Each well was measured at Abs₄₅₀ with an ELISA plate reader. Each data point was performed in duplicate and error bars represent standard deviation.

PPAb was collected from cultured B cells isolated 7 days after vaccination of healthy individuals as previously described (He et al., 2010). The volunteers were vaccinated with either inactivated 2009 pandemic H1N1 monovalent influenza vaccine (unpublished results) or 2009 seasonal trivalent influenza vaccine (TIV) (Sasaki et al., 2011). Antibody concentrations were normalized to IgG concentration for each vaccine recipient, and samples represent pooled fractions from 15-20 individual vaccine recipients. PPAb was diluted with RPMI 1640 media with 10% fetal bovine serum.

Fetuin Binding of HA Head Domain Proteins

For the fetuin binding assay, 200 μL of fetuin (Sigma-Aldrich) at 200 $\mu\text{g/mL}$ concentrations were coated on 96-well Nunc MaxiSorp plates (Thermo Scientific, Rockford, IL) and allowed to bind overnight at 4°C . Purified, soluble HA head domain proteins were precomplexed with rabbit anti-*Strep*-tag II antibody (GenScript, Piscataway, NJ) and HRP-linked anti-rabbit IgG antibody (KPL) (24:2:1 molar ratio) for 2 h at 0°C prior to incubation

of limiting dilutions on the fetuin-coated plates. HA binding was subsequently detected using tetramethylbenzidine substrate (Thermo Scientific) in a VersaMax ELISA microplate reader (Molecular Devices, Sunnyvale, CA) measuring Abs₄₅₀.

Results and Discussion

Production and Purification of HA Head Domain Monomer

To produce the HA head domain in monomeric form in CFPS reactions, the coding sequence was first designed to express residues 63-270 from the HA primary sequence, and the DNA sequence was optimized for *E. coli* production using DNAWorks (Hoover and Lubkowski, 2002) in order to achieve optimal translation rates in the *E. coli*-based CFPS system. The DNA sequence was further optimized at the 5' end to discourage formation of secondary structure using M-fold (Zuker, 2003), and the coding sequence for a C-terminal *Strep*-tag II peptide was added. More than 40% of the 340 µg/mL of CFPS produced HA head domain was soluble when expressed at 25 °C for 12 h (Table I). The protein was readily purified with *Strep*-Tactin resin (IBA GmbH) (Fig. 1).

Production and Purification of HA Head Domain Trimer

To induce trimerization of the HA head domain, the 29 amino acid foldon domain from the T4 phage fibritin protein, which has previously been used for effective trimerization of other protein constructs (Tao et al., 1997; Frank et al., 2001; Stevens et al., 2004; Ekiert et al., 2009), was fused to the C-terminus of the HA head domain. Initial attempts to produce this construct in CFPS yielded mostly insoluble products, and when purification using *Strep*-Tactin resin was performed, very little HA head domain protein was recovered (Table I, Fig. 2A). The majority of the purified product consisted of the GroEL chaperonin complex, presumably a co-purification product associated with misfolded HA head domain. Since GroEL seemed to be an important chaperone in the folding pathway of HA head domain trimer, the HA construct was further tested in CFPS reactions with extracts overexpressing the GroEL/S chaperonin complex. While this CFPS method allowed for much better purification yields of the HA head domain, it did not mitigate the co-purification of GroEL (Fig. 2B), and a further purification step was necessary for separation of the two products.

To better produce the trimeric HA head domain protein with higher soluble yields and without the need for multiple purification steps to remove co-purified GroEL, a combination of mutations was made to the construct containing the foldon domain. A set of three consecutive isoleucine residues occurs at the C-terminus of the HA head domain sequence. These hydrophobic residues come in close contact with the stem domain in the full-length ectodomain structure but are probably exposed when the head domain is expressed alone. While these residues did not appear to have a dramatic effect on the folding of the monomer structure, in the trimer form, where the C-termini come in close contact with one another and the propensity for aggregation is increased, the residues may exert a stronger deterrent to correct folding. Thus, the consecutive isoleucine sequence (III) was mutated to more hydrophilic serine and glycine residues (SGS), and the construct produced higher soluble protein yields (Fig. 3). A second, serendipitous mutation of a methionine (M260) to a leucine was discovered while creating methionine mutations to the HA head domain

sequence for separate applications. While this mutation alone also increased soluble yields of trimeric HA head domain, more soluble protein was produced when it was combined with the mutated isoleucine sequence with 180 µg/mL of the 230 µg/mL total produced as soluble protein (Fig. 3). When this construct with both sets of mutations was purified using *Strep*-tag affinity, high yields of soluble protein were recovered with little co-purification of GroEL. GroEL in the final pooled protein was further minimized by not including the first few elution fractions where most of the GroEL was present (Fig. 2C).

Characterization of HA Head Domain Proteins

To determine if the HA head domain trimer construct containing stabilizing mutations was correctly folded and assembled, the purified protein was compared to the monomer construct using size-exclusion HPLC (Fig. 4). Both proteins eluted as strong single peaks with minimal aggregated protein eluting in the void volume (at approximately 10.5 min). The clear shift to an earlier elution peak time for the trimeric construct (13.5 min) compared to the monomeric construct (16 min) indicates that the protein forms a multimer with the addition of the foldon sequence, presumably in the trimer conformation based on the spacing between the peaks compared to molecular weight standards. Both proteins eluted slightly later than might be expected by their molecular masses compared to molecular weight standards, suggesting a compact structure and a smaller dynamic radius for both forms of the head domain (data not shown).

To further confirm the structural integrity of the stabilized HA head domain trimer, the construct was compared with the monomer using an ELISA binding assay. The proteins were coated on high-binding ELISA plates and then probed with plasmablast-derived polyclonal antibodies (PPAb) from volunteers that had been vaccinated with either the 2009 pandemic H1N1 influenza vaccine or the seasonal trivalent influenza vaccine (TIV). The trimer construct bound more antibodies from the pandemic H1N1 vaccine recipients and at higher dilutions than the monomer construct (Fig. 5). This would be expected given that any antibodies binding conformational epitopes requiring multiple subunits of the HA homotrimer would only bind the HA head domain trimer and not the monomer conformation. Antibodies from pandemic H1N1 vaccine recipients also bound to more of the HA head domain constructs than did antibodies from seasonal TIV vaccine recipients for both trimer and monomer constructs, possibly because the PPAb from the pandemic H1N1 vaccine recipients recognize additional epitopes specific for the H1 strain that was used to produce the HA head domain proteins (Fig. 5).

Both of the CFPS purified constructs bound significantly less antibody than commercial full-length HA ectodomain produced in mammalian cells (Immune Technology Corp.) (Fig. 5). This was expected since the commercial HA had more immunogenic epitopes from both the stem domain and from any glycosylation compared to the aglycosylated CFPS-purified HA head domain proteins. The ELISA analysis nevertheless suggests that the trimer construct of the HA head domain protein is properly folded and provides more antibody epitopes than the monomer.

The monomer and trimer HA head domain preparations were also assessed for binding to terminal sialic acid sugar residues using an ELISA plate assay. In this case, the glycoprotein,

fetuin, which contains terminal sialic acids, was bound to ELISA plates and probed for binding with the HA head domain preparations. The trimer demonstrated stronger binding as compared to the monomer, while the monomer still demonstrated significantly more binding than green fluorescent protein (GFP) which was used as a negative control (Fig. 6). This assay further indicates that the HA head domain trimer is properly folded and also shows improved protein functionality compared to the monomer. The data also confirm that the stabilizing mutations made to the trimer construct do not interfere with sialic acid binding as anticipated since all of the mutations were made to residues distal to the sialic acid binding site.

Conclusions

In this paper, we describe a method for producing HA head domain protein from the 2009 pandemic H1N1 influenza strain in either monomeric or trimeric form using *E. coli*-based CFPS. The CFPS produced protein abrogates the need for refolding that has been required with previous production of HA constructs *in vivo* in *E. coli* (Song et al., 2008; Song et al., 2009; Aguilar-Yanez et al., 2010; Alvarez et al., 2010; Khurana et al., 2010) and allows for single-step affinity purification. We also, for the first time, demonstrate efficient production of the assembled trimeric head group. The purified trimeric HA head domain protein showed more antigenic epitopes and better sialic acid binding compared to the monomeric protein, which is expected given that the trimeric protein more closely resembles the native HA conformation, and highlights the structural and functional differences of the constructs. The CFPS platform also provides a means to combat pandemic threats when a rapid response with large amounts of protein is required.

In future studies, the immunogenicity of the HA head domain proteins could further be increased in fusions or conjugations with other immunostimulatory species. HA head domain fusions with the flagellin protein have previously been described (Song et al., 2009). CFPS has also been shown to be a viable method for introducing non-natural amino acids that could facilitate covalent attachment of these antigens to other proteins (Patel et al., 2009; Bundy and Swartz, 2010) or to multiple immunostimulatory species using virus-like particles (VLPs) as an attachment platform (Patel and Swartz, 2011). The HA head domains could also be used in assays to help detect emerging viral strains where quick evaluations are required (Alvarez et al., 2010). The production methods described in this paper provide a means to rapidly produce useful material for combating influenza threats.

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Figure 1.

Elution fractions of CFPS produced HA head domain purified with Strep-tag II affinity tag. Elution fractions were 0.5 mL each and pooled fractions used for further characterization are enclosed by the black box.

Figure 2.

Elution fractions of CFPS produced HA head domain construct with the C-terminal foldon trimerization domain after purification using the Strep-tag II affinity tag. A) The original HA head-foldon construct yielded mostly co-purified GroEL chaperonin complex. B) Production of HA head-foldon in CFPS extracts overexpressing GroEL/S chaperonins yielded more purified HA head-foldon in addition to increased levels of co-purified GroEL. C) HA head-foldon with multiple stabilizing mutations minimizes co-purification of GroEL and greatly increases the recovered yields of HA head-foldon. Pooled fractions used for further characterization are enclosed by the black box. Elution fraction volumes were 0.5 mL for A, 0.5 mL for B, and 1 mL for C. The denaturing SDS-PAGE gels disassociate the GroEL complex as well as the HA head domain trimers, causing all gel products to run as monomers.

Figure 3. Stabilizing mutations made to the HA head-foldon construct. Three isoleucines (III) near the C-terminus of the HA head domain were mutated to serines and glycines (SGS), and a methionine (M260) was mutated to leucine. The mutations increased soluble yields in CFPS alone and further increased soluble yields in combination. All reactions were performed in triplicate, and error bars represent standard deviation.

Figure 4.

Size-exclusion HPLC to assess trimerization of HA head domain proteins. The HA head domain elutes as a single peak with a retention time of approximately 16 min. The inclusion of the C-terminal foldon domain induces trimerization with this HA head-foldon construct eluting with a retention time of approximately 13.5 min. Small peaks at 10.5 min represent aggregates.

Figure 5.

ELISA binding of antibodies to HA head domain proteins. HA head domain protein preparations based on the 2009 pandemic H1N1 strain were coated on 96-well plates and probed with plasmablast-derived polyclonal antibodies (PPAb) from volunteers vaccinated with either 2009 pandemic H1N1 monovalent vaccine (solid shapes and lines) or seasonal trivalent influenza vaccine (TIV, open shapes and dashed lines). Commercial HA was glycosylated, full-length HA ectodomain, and HA head domain monomer and trimer constructs were produced by CFPS. All HA constructs bound more antibodies from 2009 pandemic H1N1 vaccine recipients as compared to those vaccinated by seasonal TIV. All data points were obtained in duplicate, and error bars represent standard deviation.

Figure 6.

ELISA binding of HA head domain proteins to sialic acid moieties. Fetuin glycoprotein was coated on 96-well plates and probed with either monomeric or trimeric HA head domain proteins from the 2009 pandemic H1N1 strain. GFP was included as a negative control. All data points were obtained in triplicate, and error bars represent standard deviation.

Table I

CFPS yields of HA head domain monomer and trimer constructs.

HA head construct	$\mu\text{g/mL}$	
	Total	Soluble
monomer	340 ± 30	140 ± 10
trimer	250 ± 50	80 ± 0.3
trimer stabilized mutant	230 ± 30	180 ± 20

Monomer was produced in sufficient quantities for purification, whereas the trimer construct required several stabilizing mutations to increase soluble yields.