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CryoEM Structure of the Adenovirus Factor X Complex at Subnanometer Resolution

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Human adenoviruses (HAdVs) have been studied extensively for their potential as therapeutic gene delivery vectors. As relatively large viruses (capsid diameter >900Å), HAdVs are able to accommodate large segments of foreign DNA. Another favorable property for gene delivery is that HAdVs are able to transduce a broad range of cell types. In HAdV vector research it is common to deliver HAdV via the minimally invasive intravascular route. When HAdV type 5 (HAdV5) is delivered intravascularly, the liver is the primary target organ. Early intravascular delivery trials of adenovirus showed interactions with plasma proteins that control its distribution and transduction properties. One of the strongest interactions is between HAdV5 and blood coagulation factors VII, IX, and X, which target the virus to the liver *in vivo*. The molecular mechanisms by which these plasma proteins bind HAdV5 are poorly defined.

Blood factors VII, IX, and X are vitamin K-dependent serine proteases that are synthesized in the liver and circulate in plasma as zymogens that become active upon proteolytic cleavage. These coagulation factors share a common domain structure: GLA (γ-carboxylate glutamic acid), EGF1 (epidermal growth factor-1), EGF2 (epidermal growth factor-2), and SP (serine protease). A crystal structure has been determined for an active-site inhibited form of human FXa, the activated form of factor X, and a model has been built for FX, the zymogenic form (Fig. 1) [1]. Waddington *et al.* and Kalyuzhniy *et al.* have shown that the GLA domain is required for HAdV blood factor interactions [2, 3]. Moderate resolution cryoEM structures have been determined for the complex of FX with HAdV [2, 3]. Here we present a subnanometer (9Å) resolution cryoEM structure of FX complexed with HAdV5 modified to have a short-shafted fiber. This structure is based on a dataset of 1,101 cryoEM particle images collected on an FEI Polara (300kV, FEG) cryoelectron microscope. The subnanometer resolution cryoEM structure shows well defined density for FX bound to each trimeric hexon capsid protein. The FX density is particularly clear at the peripentonal hexons, which are next to penton bases at the vertices of the capsid (Fig. 2). At this site, density for FX bound in three symmetrically related orientations with respect to hexon is observed. The goal of this project is to use the subnanometer resolution cryoEM structure to guide modeling of the HAdV hexon/FX interaction [4].

References

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[4] This research was supported by an NIH grant to DMS and PLS (R01 CA141439).

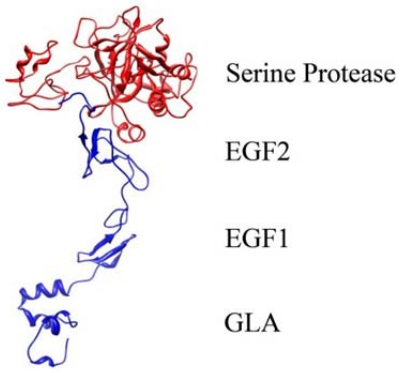

FIG. 1. Model of the zymogenic form of human coagulation factor X [1]. FX is composed of a 306-residue heavy chain (red) that is covalently linked by a disulfide bond to a 139-residue light chain (blue). The light chain of FX contains the γ-carboxyglutamic acid (GLA)-rich, and epidermal growth factor like -1 and -2 domains. The heavy chain of FX contains the serine protease domain.

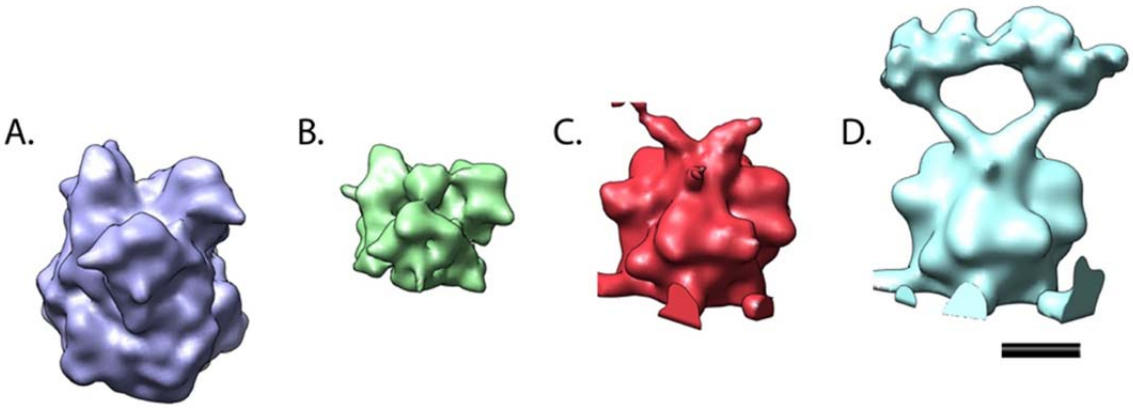

FIG. 2. Density maps of HAdV5 hexon with and without bound FX. (A) Crystal structure of the HAdV5 capsid protein hexon (PDB ID 1P30) shown as a density map filtered to 15-Å resolution. (B) The top of a peripentonal hexon from the 9-Å resolution cryoEM structure of the HAdV5/FX complex contoured to emphasize density from the FX GLA domain bound in the central depression of hexon. (C) The same hexon filtered to 10-Å resolution and contoured to emphasize two strong and one weak EGF-1 domains protruding from the GLA domain. (D) The same hexon filtered to 20-Å resolution and contoured to show density for the two most favored orientations of FX. Scale bar, 50 Å.

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