Structural Studies of an Icosahedral Virus Engineered with His-Tags

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Introduction

His-tag–Ni resin is a well-established system for purification of heterologously expressed proteins. However, His-tags can also be engineered onto an icosahedral virus, Cowpea mosaic virus (CPMV), to alter the surface properties of the capsid and be exploited beyond the binding of nickel ions for new activities. Several virus mutants are made with the His-tag presented in the icosahedral constellation. As a first step in characterizing these mutants, the "mode" of His-tag–Ni binding is being investigated. The CPMV/His-tag virus particles are crystallized and subjected to structural studies.

Methods and Materials

Cubic CPMV crystals were grown under conditions previously reported [1]. The crystals were flash station cooled, and the data were collected at beamline station 14-BM-C at the APS. The different types of mutants and their complexes with Ni are shown in Table 1. DENZO was employed for the data processing [2], and RAVE is being used for the structure determination [3].

Results

His-tags are presented on multiple locations on the CPMV surface (Fig 1). Apparently, insertion of His-tags did not interfere with crystallization, and rhombic dodecahedral crystals similar to those of the wild-type virus were obtained for all mutants. Ni ions were soaked into the crystals, and the data from both the native chimeric viruses and the Ni/virus complexes were collected, when permitted. The crystals diffracted the x-ray to moderate resolutions of 3.2-3.5 Å. Table 1 shows the statistics in data processing.

There are different degrees of shrinkage in the unit cell dimensions due to flash cooling, which is common for

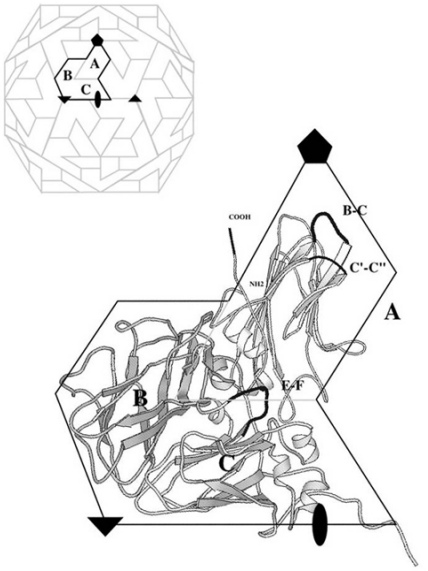

FIG. 1. Locations of His-tag presentation. Upper left: Schematic presentation of the virus capsid. The asymmetric unit is highlighted, and the symmetry elements are shown. Lower right: Ribbon diagram of the asymmetric unit. Four locations (shown in a dark shade) were exploited for insertional mutagenesis. Three of these locations are in the A domain (C-terminus, βB-βC loop, and βC′-βC″ loop), and the other is in the C domain (βE-βF loop).

CPMV crystals. The R_{sym} are high for two of the constructs (AC3/4 and AC7/8), reflecting the quality of these crystals. The data completeness of 71% to 83% should be sufficient for structure determination in consideration of the fivefold NCS redundancy. The resolutions of the data are moderate but sufficient for visualization of the His-tags if they are ordered.

TABLE 1. CPMV mutants with Ni complex and statistical results.

Chimera	Cell D (Å) (I23)	Reflections	R_{sym}	Completeness (%)	I/σ	Resolution (Å)
AC5/6 (βE-βF) native	312.0	69,159	8.2	83	13.2	3.5
AC5/6 (βE-βF) complex	311.4	61,882	7.1	78	14.2	3.3
AC3/4 (βC′-βC″) native	310.7	44,401	17	71	10.5	3.5
AC3/4 (βC′-βC″) complex	311.5	64,475	15.3	78	16.5	3.2
AC7/8 (C-term) complex	313.7	57,016	13.6	81	13.1	3.4

Discussion

The data show reasonable statistics, at least for AC5/6 and its Ni complex, and the structures are being determined. We exploit CPMV as a source of biomaterials for applications in nanotechnology [4]. Understanding of His-tag – Ni interactions in the icosahedral constellation is the prerequisite for the structure-based engineering of new binding activities on the virus surface.

References

[1] T. Lin, Z. Chen, R. Usha, C.V. Stauffacher, J.-B. Dai, T. Schmidt, and J.E. Johnson, Virology **265,** 20-34 (1999).

[2] Z. Otwinowski, Proc. CCP4 Study Weekend (1993), pp 56-62.

[3] G.J. Kleywegt and T.A. Jones, Proc. CCP4 Study Weekend (1994), pp. 56-66.

[4] Q. Wang, T. Lin, L. Tang, J.E. Johnson, and M.G. Finn, Angew. Chem. Int. **41,** 459-462 (2002).