Detailing the Quaternary structure of the virus using tritium planigraphy

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Tritium planigraphy (TP) refers to methods of examining spatial structure of biological macromolecules. The essence of the method is the bombardment of the surface by the flow of hot tritium atoms with specially selected characteristics. The modification consists in replacing ordinary hydrogen in the CA-atoms and side groups of amino acid residues (RA) on its isotope tritium, which obviously does not change the object structure. The small size of the atom of tritium (~0,09 nm) used as nano-probes allow to detect the sites localized in the surface layer of the macromolecule. Next separation of a protein to the fragments of molecule (down to individual RA) and determination of the tririum distribution allows to obtain information on the steric accessibility of the system components [1-3] and to reveal the details of the spatial structure of the object.The method of determining the orientation of the subunits (SU) of virus is proposed on the example of X-PVX potato virus of the family Alphaflexiviridae genus Potexvirus. Note that the solution of this task according TP for a single object is difficult due to the fact that the rotation of the elementary platforms around the flow direction does not change its value.

The calculations used: (i) cryoelectron microscopy data, which suggests that the PVX virions are flexible thread-like particles with a length of 515 nm. 1270 subunit of the shell form a helix (Fig.1) with a diameter D = 13.0 nm, with step H = 3.45 nm and n = 8.89 SU on turn of helix [5].

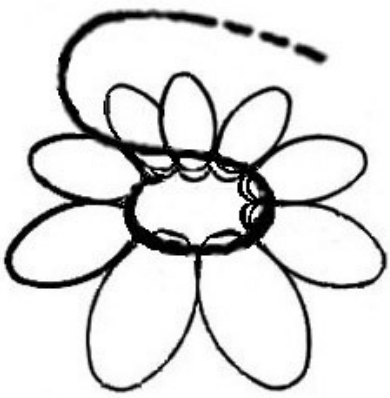

Fig.1. The scheme of packing of protein molecules in the helical structure of the virus. Shown a single turn. subunits)

The question about their orientation remains open. It is also unknown which amino acid residues in the SU included in the contact area with adjacent subunits; (ii) model file of the subunit (the coordinates of the centers of the atoms of each of the 237 amino acid residues

is obtained on the basis of homology modeling of proteins shell potexviruses and the program Modeller [4]; (iii) data tritium planigraphy (Fig.2).

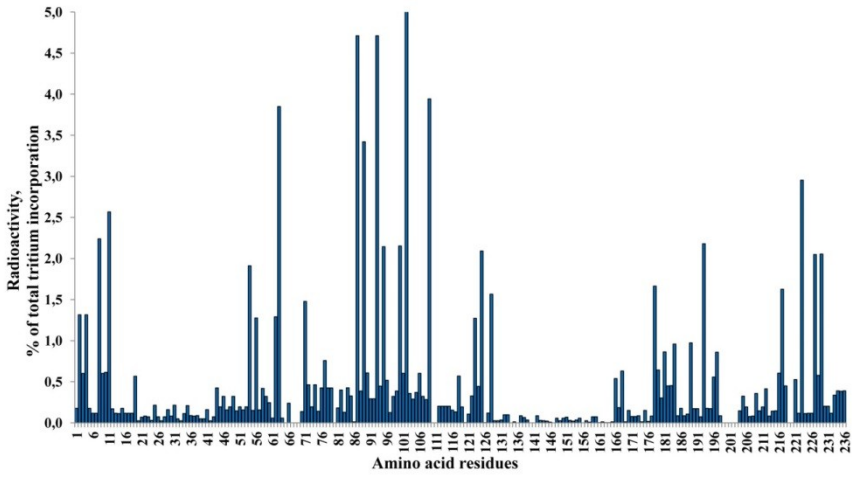

Fig.2. Inclusion of tritium label on individual amino-acid residues RAi of subunits.

Based on these data, the problem of the of finding of the subunits orientation of relative to the helical axis was solved, as close as possible to the data of TP – activity distribution of tritium labels for RA_i.

The calculation algorithm structure consists of two parts:

1. Especially previously developed algorithm for simulated bombardment of isotropic flux of tritium atoms take into account the shielding of the atom by other atoms [6].
2. A new approach to the determination of the subunits orientations due to the space of limitations.

Selection criteria orientation:

1. The best fit to the experimental data of TP on the correlation coefficient between the activities of protein amino acid residues and the calculated flow J.
2. Perform the necessary spatial constraints if the spiral stacking:

2.1. The distance to the helical axis smaller than the radius of the helix D/2 = 6.5 nm ($r_i – r_j$, EP) < D/2;

2.2. The projection on the azimuth direction is less than the chord L:($r_i – r_j$, e_φ) < L=2R*sin(π/n) = 4.5 nm

2.3. The projection on the axis of helix, the more the pitch of the helix H = 3.45 nm (r_i-r_j, e_z) > H (parameter values from [5], e unit vectors in the respective direction, the r vectors of the centers of the atoms of the subunit).

Used assumptions:

1. The proportionality is measured on the experience of the activity and the flow J, which is oriented to elementary platform dS (Fig.3) is defined as

$$J = \int j(n)(n \cdot \mathbf{dS})\, d\Omega$$

Here j (**n**) - flow distribution in a direction defined by unit vector n, $d\Omega$ - element of the solid angle corresponding to a given direction; the integration is carried out on all available directions. The calculation of J was carried out according to the previously developed method of simulating a bombing isotropic flux of tritium atoms [6].

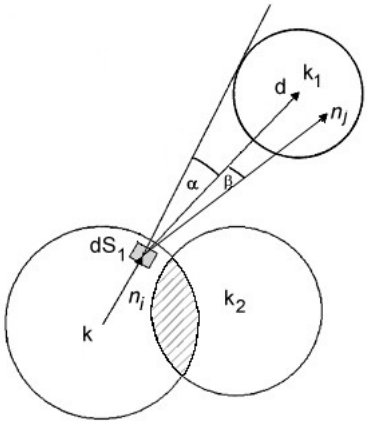

Fig.3. To the algorithm of calculating the accessible sur_face of macromolecules. On the surface of a *k*_th atom, elements *dSi* = *nidS* are chosen beyond the overlap of atoms *k* and *k*2. For dSi, directions *nj* are chosen such that *ninj* > 0. Shielding by atom *k*1 is taken into account by deleting the *nj* with α > β; α is angle between ray *d* from the *dSi* center to the *k*1 center and the tangent to *k*1, β is angle between *d* and *nj*.

2. Subunits of the virus form a helix. Each atom of one SE is transmitted to the corresponding atom in the following rotation about the helix axis by angle α = 2π/n and the ascent to H/n.

Results and discussion

The spatial constraints satisfying the above mentioned conditions were selected. Possible directions of the helical axis and rotations perpendicular to this plane axis were obtained: from 12000 options to only 122, i.e., ≈ 1 %. Flux $\mathbf{J}_i$ was calculated for the 26 most tritium labeled residues, and the comparison with experiment was carried out. The area of intersection of adjacent van der Waals spheres and the shielding of the flux from non-adjacent atoms in the flow path were taken into account. The calculated value of the correlation coefficient between the estimated flow and TP data amounted to 0.79-0.84. The most probable orientation for the subunits, approximated ellipsoid, is shown on fig. 4.

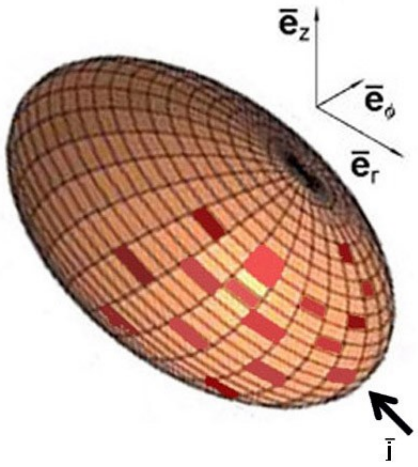

Fig.4. The best orientation of subunits in the virus. Z-direction of helix axe, $\mathbf{j}=\mathbf{e}_r$ – direction of flow.

In considering the quaternary structure of the virus for each orientation, you can define groups of residues forming the contact area with an adjacent subunits , as well as the most exposed to the flow. The residues bordering the adjacent subunits located on the upper turn of the helix, are - 10A, 11T, 12G, 13S, 14T, 15T , 104Y, 105S, 177K, 181I. Residues bordering a low turn of the helix are - 69M, 70K, 101T, 106N, 108I, 109S, 7T, 8T,and 9Q, 10A, 218T, 219R, 220G, 222I, 234L, 235P, 236P, 237P. Residues in contact with next of the previous in the course of the spiral of the SU are 32L,33F,34T,35I,37D, and 38G, 185S,186Q,187A,188Q,189M,190N,191A,193Q. For the rest, maximally exposed to the flow, it's - 104Y,105S,177K,181I, 185S,186Q,187A,188Q,189M,190N,191A,193Q.

Conclusions

1. A new technique to determine the orientation of subunits in the virus using the data of TP and the spatial constraints in a helix laying of the SU in virus, allow to determine their orientation relative to the helical axis and the axis of symmetry of the flow perpendicular to it.
2. The most common groups of the residues in adjacent subunits, forming the contact zone, are determined, that allows to detail the Quaternary structure of the virus.

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