Determination of low resolution structure of human immunoglobulin M and rheumatoud factor IgM-RF in solution.

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Antibody macromolecules present a family of structurally dynamic glycoproteins - immunoglobulins, which provide foreign body recognition and elimination processes [1]. To know the correlation between the immunoglobulin structure and its function is an important problem for immunology and medicine. In this work, the two methods were implemented to the structure investigation: atomic force microscopy (AFM) and small angle X-ray scattering (SAXS) in solution. The main advantage of the SAXS method is that it allows to study the low-resolution structure of large biological macromolecules under nearly physiological conditions in solutions. The AFM approach provides the possibility of studying the low-resolution shape of a macromolecule directly, without a mathematical reconstruction. Comparison of the AFM results with SAXS structures enhance the reliability of the final structural model and the molecular envelopes obtained from AFM experiments may be used as initial models in the SAXS methods. The SAXS intensity curves are sensitive to the overall shape and quaternary structure of the solute particles to a resolution of 2-3 nm. It is impossible to obtain directly spatial structural models from the experimental data but the scattering pattern from any three-dimensional structure could be calculated. This fact lies in the background of the method of determination of structure by dummy atom modeling. The method is based on the simulated annealing optimization procedure that minimizes the total squared discrepancy between experimental SAXS data and data calculated from the structure represented by the varying set of small spheres (dummy atoms) with the desired density arranged in the spherical search area [2]. The diameter of the search area is determined from the SAXS patterns as the maximum dimension D_{max} of the molecule . The main advantage of this approach is that there are no special restrictions on the complexity of the particle structure. The immunoglobulin molecules were represented by a single-phase model structure consisted of two kinds of beads: a solvent and a particle. A five-order symmetry axis was assumed both for IgM and IgM-RF

models. The synchrotron radiation X-ray scattering data were collected following standard procedures using X33 camera of the European Molecular Biology Laboratory (EMBL) on the storage ring DORIS III, DESY, Hamburg. The radii of gyration of molecules were evaluated as 12.1±0.2 nm for IgM and 10.9±0.2 nm for IgM-RF from the characteristic functions using the indirect transform program GNOM [3]. This program was also used to estimate the distance distribution functions and the maximum dimensions D_{max} of the dissolved molecules (36.5±0.5nm both for IgM and IgM-RF). Structure determinations were performed using the dummy atom modeling program DAMMIN [2]. The dummy atom structures of IgM, IgM-RF, and the atomic model proposed earlier by Perkins [4] are shown in the Fig.1a-c. Fab regions of IgM-RF model are asymmetric which corresponds to the assumption of dissimilarity of the Fab regions supposed earlier [5]. Five-order symmetry was forced in the dummy atom models as was mentiond above which is reasonable for the IgM structure. This symmetry is used in the case of IgM-RF as an approximation because the ratio between "full" and "reduced" Fab fragments was found earlier [5] as 6:4 which is close to 5:5.

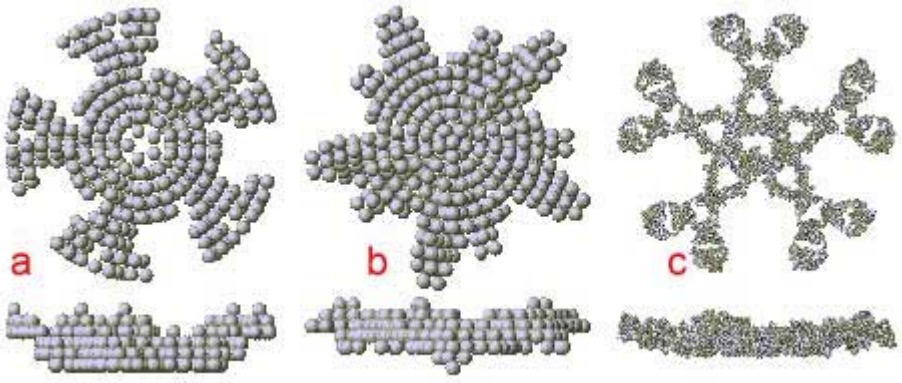

Fig 1. Restored dummy atom structures of immunoglobulins IgM(a), rheumatoid factor IgM-RF(b), and proposed in [4] atomic model of IgM (c).

Samples for AFM investigations were prepared by imposing of molecules from solution (concentration 0,006 mg/ml) on fresh cleavage of mica. AFM investigation of samples was carried out in air using the atomic force microscope P47-SPM-MDT (Russia, NT-MDT) in tapping mode. Si cantilevers (NSC11, Mikromasch, Estonia) were used. The AFM images also show the difference between envelopes of IgM and IgM-RF (Fig.2, fig.3).

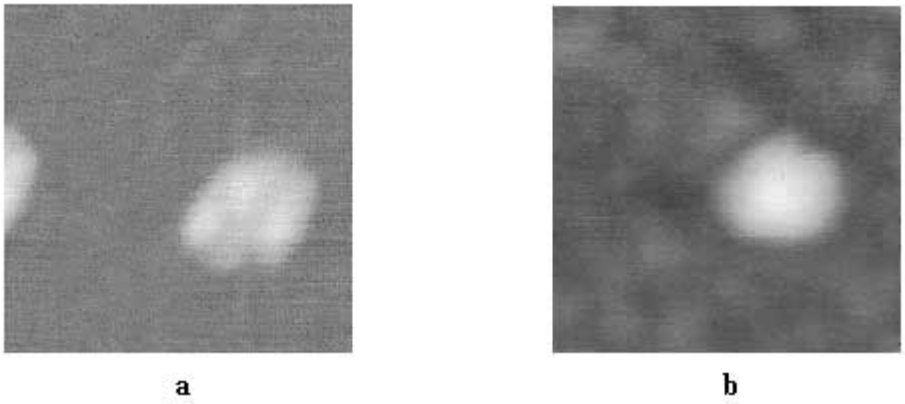

Fig. 2. AFM images of IgM (a) and IgM-RF (b). Size of both images 70×70 nm.

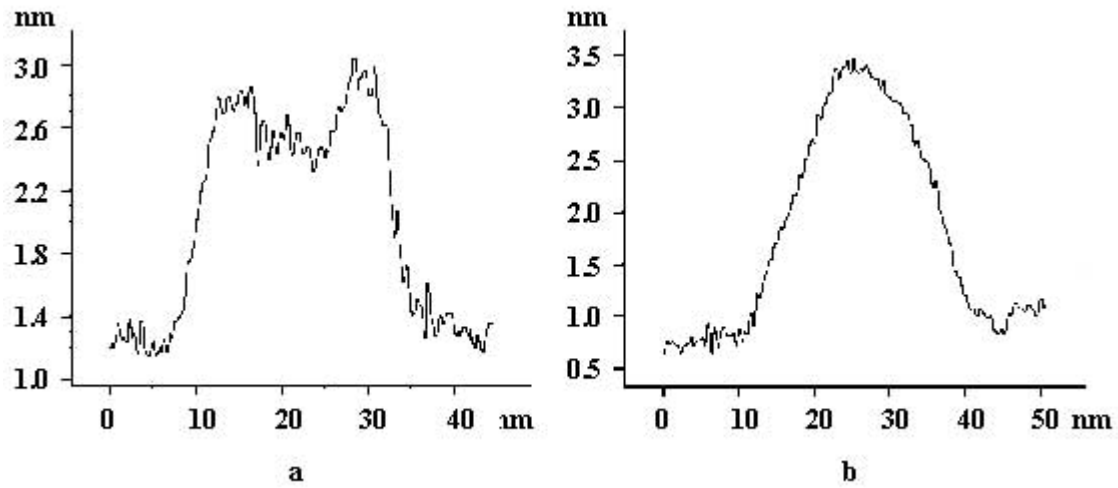

Fig. 3. Profiles from AFM images of IgM (a) (along X axis through 25 nm) and IgM-RF (b) (along X axis through 32 nm).

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