Eur Biophys J (2001) 30: 457–462
DOI 10.1007/s002490100176

ARTICLE

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HYDROMIC: prediction of hydrodynamic properties of rigid macromolecular structures obtained from electron microscopy images

Received: 24 April 2001 / Revised version: 14 June 2001 / Accepted: 14 June 2001 / Published online: 28 August 2001

Abstract We have developed a procedure for the prediction of hydrodynamic coefficients and other solution properties of macromolecules and macromolecular complexes whose volumes have been generated from electron microscopy images. Starting from the structural files generated in the three-dimensional reconstructions of such molecules, it is possible to construct a hydrodynamic model for which the solution properties can be calculated. We have written a computer program, HYDROMIC, that implements all the stages of the calculation. The use of this procedure is illustrated with a calculation of the solution properties of the volume of the cytosolic chaperonin CCT, obtained from cryoelectron microscopy images.

Keywords HYDROMIC · Hydrodynamic coefficients · Solution properties · Macromolecules · Electron microscopy

Introduction

Hydrodynamic coefficients and other solution properties are useful sources of information on the solution structure of macromolecules and macromolecular complexes. Their utility has been classically appreciated (Tanford 1961; Daune 1998; van Holde 1998), and it is presently enhanced after the advent of modern instrumentation for analytical ultracentrifugation (Harding et al. 1992) and a variety of dynamic spectroscopic techniques. The theoretical and computational procedures, obviously required to relate structure and properties, and particularly the bead models pioneered by Bloomfield et al. (1967), have been developed (Garcia de la Torre and Bloomfield 1981; Garcia de la Torre 1989; Carrasco and Garcia de la Torre 1999), benefiting from advances in hydrodynamic theory and the unceasing increase in computing power.

Along the years, our knowledge of macromolecular structure has become more extensive and accurate. This has motivated further efforts in hydrodynamic theory and computation, which was initially intended for low-resolution modelling (Garcia de la Torre et al. 1994). Recently, implementations of the hydrodynamic bead modelling procedures have reached the atomic level. New computational tools like HYDROPRO (Garcia de la Torre et al. 2000a) and HYDRONMR (Garcia de la Torre et al. 2000b) allow the prediction of hydrodynamic coefficients and NMR relaxation times, and the program SOLPRO (Garcia de la Torre et al. 1999) is also available for calculation of scattering and other solution properties. These tools are intended for the prediction of properties from the atomic structures of proteins and nucleic acids as specified by atomic coordinates from crystallographic or NMR structures deposited in the PDB (Protein Data Bank) or similarly formatted files.

During the last decade, electron microscopy has become a major tool in macromolecular structure determination. The intensive use of cryoelectron microscopy and the development of more powerful image methods of quantitative image analysis have extended the use of electron microscopy towards high resolution. Presently, electron microscopy is capable of providing not only information of proteins at atomic resolution (Henderson et al. 1990; Kühlbrandt and Wang 1991; Nogales et al. 1998) but also of generating medium-high resolution information of large macromolecular complexes that

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are difficult to crystallize, and which can be later used to dock the atomic structure of their basic constituents (Kalko et al. 2000) to obtain very useful biological information in this way.

In this work we have developed the methodology that enables the prediction of solution properties from the 3D reconstructed images derived from electron microscopy and related techniques, using bead-model calculations. Different varieties of bead modelling have been described (Carrasco and Garcia de la Torre 1999), so it has been necessary to find the proper way of doing such modelling in a way that starts directly from the 3D image formats that are usually produced by those techniques.

As a test for the methodology, we have used the 3D reconstruction of the cytosolic chaperonin CCT, obtained using cryoelectron microscopy and image processing. Chaperonins are a group of proteins that are involved in the assistance to the folding of other proteins. They form large macromolecular complexes built by two rings, each one having 7–9 subunits, depending on the chaperonin, and with a total molecular weight ranging from 840 to 1080 kDa. CCT is the only chaperonin found in the eukaryotic cytosol and is a very unique chaperonin in the sense that is much more complex than any other chaperonin known, and that it functions in the folding of a very small number of proteins, especially actin and tubulin, whose importance in the cell function is paramount (Llorca et al. 1999a, 1999b, 2000).

Method

HYDROMIC

The first part of the methodology has the purpose of building a suitable hydrodynamic model from the 3D shape of the particle, and this has to be done according to the contents and format of the computer files that contain the shape information. A common feature of some of these files is that they are based on a cubic grid, which is superimposed on the particle. In the file, the nodes of the grid, usually named pixels, are assigned numerical values. The number of segments in which the x, y and z dimensions are divided are, respectively, N_x (number of rows), N_y (number of columns) and N_z (number of slices). The spacing, b, is the same for the three axes. The various file formats differ in how this and other information is organized and coded. Here we consider the so-called spider format (Frank et al. 1996), which is one of the most frequently employed in the electron microscopy field.

Files in the spider format (see the web sites http://www.cnb.uam.es/~bioinfo/NewXmipp/Extra_Docs/FileFormats.-html and http://www.wadsworth.org/spider_doc/spider/docs/image doc.html, and Frank et al. 1996) contain a header within which the values of N_x, N_y and N_z are given. The total number of pixels in the file is $N_{tot} = N_x N_y N_z$. Then there is a list of values, one for each pixel, whose values correspond to their level of density. For our present purpose, we consider that a pixel belongs to the particle if the value, v, assigned to it exceeds some threshold, $v_{\min}$. The values of b and $v_{\min}$ have to be supplied separately. Our HYDROMIC program reads the spider format, selects the pixels that belong to the particle and calculates their Cartesian coordinates.

After processing the structural file, HYDROMIC has a list of coordinates of N_p points, or pixels, which fills the particle contour. Some basic geometric information is determined at this stage; thus, the particle's volume can be estimated as $V = b^3 N_p / N_{tot}$ and the radius of gyration, R_g, can be readily calculated from the pixel coordinates. From this list of coordinates, a primary hydrodynamic model (PHM), composed of spherical elements, is obtained by replacing each pixel by a sphere. The radius of the spheres, a, whose precise value is not essential for the final results, is selected in such a way that each sphere overlaps appreciably with its neighbours, so that there will be no interstitial voids in the model. A proper choice is $a = b$.

Once HYDROMIC has built the PHM, the calculation of its hydrodynamic properties is carried out following the shell-limit strategy (Carrasco and Garcia de la Torre 1999). The PHM is first replaced by a hexagonal, closest-packed array of non-overlapping spheres (''minibeads'') of radius σ, thus obtaining a filling model, and then all the internal beads are removed to obtain the shell model, which is the one on which the hydrodynamic calculations are done using the HYDRO subprogram (Garcia de la Torre et al. 1994). Such calculations are repeated for several values of σ, and for each property the results are extrapolated to $\sigma = 0$. Other solution properties can be calculated from the bead models using procedures coded in our SOLPRO program (Garcia de la Torre et al. 1997, 1999). The basic procedures developed to deal with electron microscopy data can be applied also to X-ray, neutron or light scattering. Therefore, we have included in HYDROMIC the calculation of the scattering form factor and the distribution of distances.

Some elementary physical data of solute and solvent are required for the calculation of solution properties: temperature T (e.g. $T = 293$ K); solvent viscosity η (e.g. $\eta = 0.01$ poise for aqueous solution); solution density, ρ (approximately $\rho = 1.0$ for dilute aqueous solution); solute specific volume, $\bar{v}$; and molecular weight of the particle, M. The radius of gyration and scattering-related quantities are essentially independent of these data. Diffusion coefficients and relaxation times depend only on T and η. The intrinsic viscosity, $[\eta]$, requires the value of M, and for the sedimentation coefficient, s, the values of M and the buoyancy factor $(1 - \bar{v}\rho)$ are required. Values of properties calculated by HYDROMIC for some given values of these physical constants can be recalculated manually for other values. Formulas for the changes produced by η and T can be found in textbooks. If the molecular weight and/or the bouyancy factors are changed to, say, M' and $(1 - \bar{v}\rho)'$, then the sedimentation coefficient and the intrinsic viscosity change as $s' = sM'(1 - \bar{v}\rho)'/M(1 - \bar{v}\rho)$ and $[\eta]' = [\eta]M/M'$.

Results

3D reconstruction of the cytosolic chaperonin CCT

Cytosolic chaperonin CCT was chosen to illustrate the HYDROMIC method because its 3D reconstruction of the chaperonin, using frozen-hydrated specimens, has been recently accomplished in our laboratory. For details, see Llorca et al. (2000).

Application of HYDROMIC to the cytosolic chaperonin CCT

We have tested HYDROMIC using the volume obtained from the 3D reconstruction of the cytosolic chaperonin CCT, using images of frozen-hydrated images of this specimen. The chaperonin used in this study is the eukaryotic type II cytoplasmic chaperonin containing TCP-1 (CCT). CCT is a heteromeric structure made of two rings, each one built up by eight different,

albeit homologous, subunits (Willison 1999) and has an overall cylindrical shape (150×160 Å; Llorca et al. 1999a). The 3D structure obtained by cryoelectron microscopy (Fig. 1; Llorca et al. 2000) has a great similarity with the X-ray structure obtained for the thermosome (Ditzel et al. 1998), a homologous chaperonin found in *Thermoplasma acidophilum*. Binding of ligands and substrates triggers large conformational changes and modifies the architecture of CCT (Llorca et al. 2000). From a hydrodynamic point of view, this structure presents very interesting features, such as a cavity in each of the two rings, and 16 lateral windows that make the central part of the structure accessible to the solvent (Fig. 1). Owing to its size and the characteristic shape, this oligomer is a good candidate for hydrodynamic modelling (Walters et al. 2000).

The structure of apo-CCT (i.e., the chaperonin without any ligand or substrate bound) was coded in a spider format file (apo_cct.spi). The spacing is $b = 3.9$ Å, and the threshold for intensities is at $v_{\min} = 0.37$ (threshold value which generates a mass with a molecular weight similar to that of the chaperonin). This file is read by HYDROMIC, which extracts the following parameters: $N_x = 70$, $N_y = 70$ and $N_z = 70$, with $N_{\text{tot}} = 343{,}000$. Using the above-mentioned threshold, the total number of pixels considered to represent the particle is $N_p = 20{,}445$. Thus, the volume of the structure is estimated to be 1.21×10^6 Å^3. If we consider a specific volume of 0.738 cm^3/g, the mass of the protein should be approximately 980 kDa, which fits quite well with the theoretical molar mass of CCT, calculated from the sequence of all eight subunits that make each of the two rings. Figure 2 reproduces the input data file for HYDROMIC.

A technical detail that deserves some comment is the replacement of the PHM by a shell model. The number of minibeads in the shell model increases as their radius, σ, is decreased, as required by the shell-model limit extrapolation (Carrasco and Garcia de la Torre 1999). The HYDRO calculations are feasible for models with up to $N_b = 2000$–3000 minibeads, and this places a lower bound for σ; in the example of chaperonin CCT this is about $\sigma = 5$ Å. The value of σ can be regarded as the resolution of the hydrodynamic model, and b as that of the original image. Then, for large macromolecular structures, determined with high resolution (small b, very large N_p) as is the case in the example of CCT chaperonin, we notice that the resolution in the hydrodynamic model (larger σ, smaller N_b) is lower, although the main features of the particle are preserved as is clearly shown by the visual comparison of Figs. 1 and 3.

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CCT CHAPERONIN        Title
apo_cct.res           Main results filename
apo_cct.spi           SPIDER filename
0.37                  Threshold in SPIDER file
3.9                   Spacing in SPIDER file
apo_cct.pri           Primary hydrod. model (RASMOL file)
7                     Number of SIGMAs
5.0                   SIGMAmin
8.0                   SIGMAmax
293.                  Temperature, Kelvin
980000.               Molecular weight
0.010                 Solvent viscosity
0.738                 Specific volume of macromolecule
1.01                  Solution density
50                    Intervals for distrib. of distances
50                    Values of H
1.E+07                Hmax (cm^-1)
*                     END OF FILE
```

Fig. 2 Printout of the input file of the HYDROMIC calculation for apo_cct

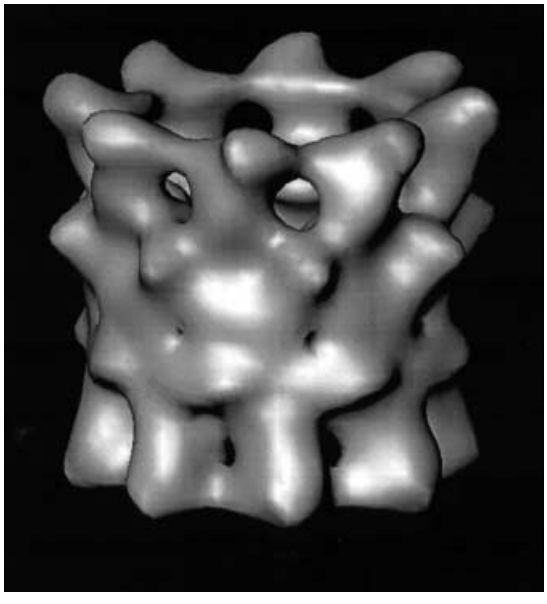

Fig. 1 Three-dimensional structure of the cytosolic chaperonin CCT obtained from image processing of frozen-hydrated specimens, as described in Llorca et al. (2000). The file with the sections of this volume (apo_cct.spi) constitutes the structural input file for HYDROMIC

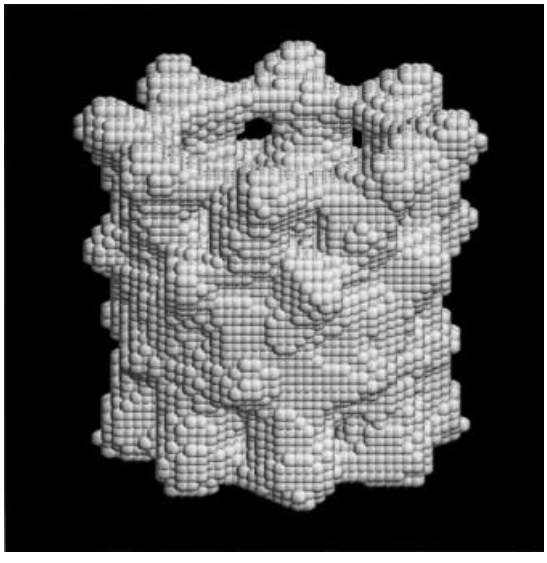

Fig. 3 Primary hydrodynamic model (PHM) of the CCT chaperonin

However, this is in part compensated by the calculations for varying σ followed by the shell-limit extrapolations to $\sigma = 0$ (we also recall that the finest details of the structure will have practically no effect on the solution properties). An appropriate checking of the performance of this procedure can be done by comparing the value of R_g of the (filling) hydrodynamic model with that previously obtained for the original structure. In the present example, we even notice (Fig. 4) that the calculated properties are quite insensitive to σ, so that the extrapolation is safe.

Figure 5 shows a reproduction of the output file from HYDROMIC, containing the results for the hydrodynamic coefficients and other solution properties. Our program also provides the distribution of intramolecular distances, $p(r)$, and the scattering function (or form factor), $P(h)$, with $h = (4\pi/\lambda)\sin(\theta/2)$ being the scattering variable for observation angle θ with radiation wavelenght λ (Glatter and Krakty 1982). In Fig. 5, the lines corresponding to these functions are removed, but a plot of them is shown in Fig. 6. It is interesting to note the skewness of $p(r)$ towards the long distances, and the early minimum in $P(h)$. Both features are probably associated with the hollow structure of the chaperonin.

Little information is available about solution properties of CCT chaperonin. Melki et al. (1997) have reported a sedimentation velocity experiment that was somehow complicated by the presence in the sample of a broad distribution of materials with different sedimentation velocities. Anyhow, these authors assigned a sedimentation coefficient of 25.6 S (water, 20 °C) to ADP-bound CCT. The value calculated by HYDROMIC using the volume of CCT obtained in the 3D electron microscopy reconstruction is 24.1 S (Fig. 5), which deviates only 5% from that mentioned above. In addition to range of accuracy of bead-shell model predictions (2–3%), the uncertainty of the experimental data and other minor effects from solvent or temperature could account for the remaining difference. Indeed, the agreement is rather good, especially when electron microscopy studies show that ADP, unlike what happens with ATP, is not able to generate large conformational changes in the chaperonin, and therefore

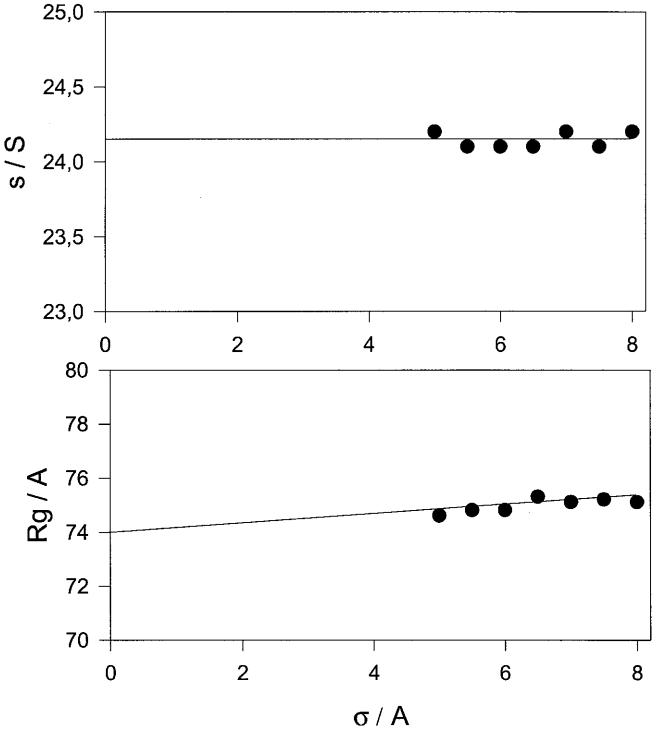

Fig. 4 Variation of the calculated properties with the minibead radius, s, showing the shell-limit extrapolations for the radius of gyration and the sedimentation coefficient. *Data points* correspond to several runs of HYDROMIC, with various values of s, and the *straight lines* are the least-squares fit used for extrapolations

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                    SUMMARY OF DATA AND RESULTS

                       This file: apo_cct37.res
                            Case: CCT CHAPERONIN (0.37
                            Mode: 31
                  Structural file: apo_cct.spi

          Data/results from spider:
                          Thresold: 0.37
                          Spacing :  3.9 A
        Nslices ; Nrows ; Ncolumns :   70 70 70
              Number of (+) pixels :   20445
                           Volume :  1.213E+06 A^3
                Radius of gyration :   74.0 A
                  Longest distance :  216.8 A

                     Molecular weight: 980000. Da
                          Temperature:  293.0 K
                    Solvent viscosity:  0.010 poise
               Partial specific volume:  0.738 cm3/g
                      Solvent density:  1.010 g/cm3
                      Bouyancy factor:  0.255
                Radius of PHM elements:  3.9 A
   Translational diffusion coefficient:  2.352E-07 cm2/s
         Stokes (translational) radius:  9.124E-07 cm
                   Radius of gyration:  7.390E-07 cm
      Rotational diffusion coefficient:  1.993E+05 s-1
                   Relaxation time (1):  8.787E-07 s
                   Relaxation time (2):  8.569E-07 s
                   Relaxation time (3):  8.569E-07 s
                   Relaxation time (4):  7.973E-07 s
                   Relaxation time (5):  7.973E-07 s
   Harm. mean relax.(correlation) time:  8.361E-07 s
                   Intrinsic viscosity:  4.861E+00 cm3/g
             Sedimentation coefficient:  2.410E+01 svedberg


                Distribution of distances, p(r)

                         r       p(r)
                    2.168E+00  5.921E-05
                    6.503E+00  2.333E-04
                    .........  .........
                    2.103E+02  3.801E-06
                    2.146E+02  4.989E-07


          CALCULATION OF SCATTERING FORM FACTOR, P, VS. H

                       H              P

                  0.00E+00       1.00E+00
                  2.04E+05       1.00E+00
                  ........       ........
                  9.80E+06       1.56E-03
                  1.00E+07       1.92E-03
```

Fig. 5 Printout of the output file of the HYDROMIC calculation for CCT chaperonin (some lines removed for brevity)

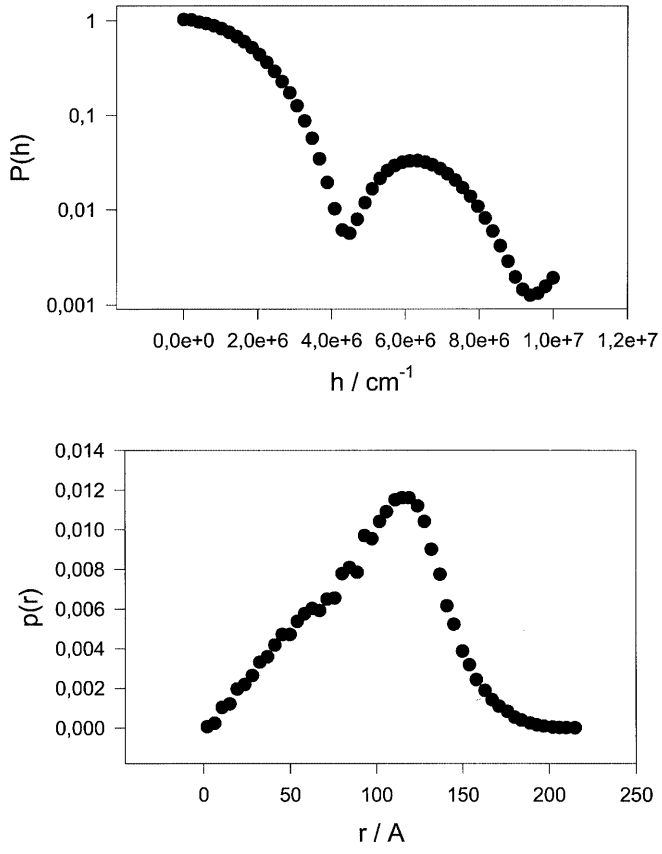

Fig. 6 *Top*: distribution of intramolecular distances, $p(r)$; *bottom*: scattering function, $P(h)$, obtained for CCT chaperonin

maintains its structure in the same conformation as the apo-CCT used in this study (Llorca et al. 1998).

Concluding remarks

HYDROMIC, a program that predicts the hydrodynamic properties of rigid macromolecular structures obtained from electron microscopy images, has been developed. The program has been tested with a large macromolecular complex, the chaperonin CCT, and the results obtained fit with the theoretical value of the molecular mass. The program opens the possibilities of predicting not only the hydrodynamic properties of a given macromolecule, but also the changes in these properties that certain ligands or substrates induce upon their binding. The experimental test of such predictions, using analytical centrifugation, low-angle X-ray or low-angle neutron scattering, can be useful for the support of the structural transitions defined by indirect experimental methods.

HYDROMIC will be freely available, along with our other computer programs, from our web site: http://leonardo.fcu.um.es./macromol/.

Acknowledgements This work was supported by grants BQU2000-0229 from the Ministerio de Ciencia y Tecnología to J.G.T., PB96-0818 to J.L.C. and PB97-1225-C02 to J.M.V.

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