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A COMPUTER PROGRAM FOR EVALUATING THE HYDRODYNAMIC PARAMETERS OF A MACROMOLECULE IN SOLUTION FOR ANY GIVEN VALUE OF ITS AXIAL DIMENSIONS*

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Abstract—A FORTRAN IV algorithm is given for determining the hydrodynamic parameters of a macromolecule in solution for any specified value of the two axial ratios (a/b, b/c) of the equivalent tri-axial ellipsoid model of semi-axes $a \geq b \geq c$ for its gross conformation.

Ellipsoid model Axial ratios Elliptic integrals Myoglobin

1. INTRODUCTION

The ellipsoid of revolution (i.e. an ellipsoid with two equal axes) has up to now been the most popular model for the gross conformation of biological macromolecules in solution. This has been until recently the most general model available for which it is possible to predict from a macromolecule's known hydrodynamic properties the shape in terms of its axial ratio without assumptions concerning hydrodynamic volume.

Although prediction of the two axial ratios from knowledge of the hydrodynamic parameters of the more general tri-axial ellipsoid presents great problems [1] the converse is not so difficult: it is relatively easy to predict the hydrodynamic parameters corresponding to a given value of the axial ratios. This would have immediate application in, for example, predicting the properties of proteins whose dimensions are known from crystallographic studies.

This paper presents a program which calculates the hydrodynamic parameters for specified values of the two axial ratios (a/b, b/c) of a general ellipsoid, where a, b, c are the three semi-axes and $a \geq b \geq c$. For an oblate ellipsoid of revolution $a = b$ and for a prolate ellipsoid $b = c$.

2. THE HYDRODYNAMIC SHAPE PARAMETERS

These can be divided into three basic classes:

(i) The viscosity increment ν. This is based on the effect of the dissolved macromolecule on the bulk motion of the fluid when a shear gradient is applied.

(ii) The translational frictional ratio f/f_o. This concerns the resistance to motion of a macromolecule through its solution when some form of external force (e.g. centrifugal field, diffusion gradient) is applied.

(iii) The rotational frictional ratios ζ_i/ζ_o ($i = a, b, c$; "o" refers to the corresponding coefficient for a hydrated sphere of the same volume) concerns the disorientating effect on the macromolecule by the local Brownian motion of the surrounding solvent molecules. Related to these are three dielectric dispersion relaxation ratios ρ_i/ρ_o corresponding to rotation of a

*This work was completed whilst the author was at the Department of Biochemistry, Leicester University, England.

particular axis about the other two, a harmonic mean relaxation time ratio τ_h/τ_o, five fluorescence anisotropy relaxation time ratios T_j/T_o ($j = 1 \rightarrow 5$) and two reduced birefringence decay constants, θ_+^{red} and θ_-^{red}.

All the above parameters require for their experimental determination a knowledge of the swollen macromolecular volume due to solvent association or "swelling" in solution. This can be eliminated by combining pairs of the above parameters to give "derived" shape parameters which are volume independent: β, R, δ_i, γ_i, μ_i, Ψ, Λ, δ_+, δ_-, γ_+ and γ_-. Each of these derived parameters is defined in Table 1 in terms of the parameters listed in Sections (i)–(iii). The relation of all these parameters with axial ratio of a general ellipsoid ($a \geq b \geq c$), is given in Table 1. Relations with experimental parameters have been given in [1, 2].

Table 1. Hydrodynamic parameters: mathematical formulation for tri-axial ellipsoids

Function	Mathematical formulation	Computer symbol
ν	$\frac{1}{abc}\left\{\frac{4(\alpha_7+\alpha_8+\alpha_9)}{15(\alpha_8\alpha_9+\alpha_9\alpha_7+\alpha_7\alpha_8)}\right.$ $+\frac{1}{5}\left(\frac{\alpha_2+\alpha_3}{\alpha_4(b^2\alpha_2+c^2\alpha_3)}+\frac{\alpha_3+\alpha_1}{\alpha_5(c^2\alpha_3+a^2\alpha_1)}+\frac{\alpha_1+\alpha_2}{\alpha_6(a^2\alpha_1+b^2\alpha_2)}\right)$ $*$ $\left.-\frac{1}{5}\left(\frac{\left[\frac{a^2-b^2}{a^2\alpha_1+b^2\alpha_2}+\frac{b^2-c^2}{b^2\alpha_2+c^2\alpha_3}+\frac{c^2-a^2}{c^2\alpha_3+a^2\alpha_1}\right]^2}{\left[\frac{a^2+b^2}{a^2\alpha_1+b^2\alpha_2}+\frac{b^2+c^2}{b^2\alpha_2+c^2\alpha_3}+\frac{c^2+a^2}{c^2\alpha_3+a^2\alpha_1}\right]}\right)\right\}$	NU
f/f_o	$2/\{(abc)^{1/3}\alpha_{10}\}$	F
ζ_a/ζ_o	$2(b^2+c^2)/\{3abc(b^2\alpha_2+c^2\alpha_3)\}$	CA
ζ_b/ζ_o	$2(c^2+a^2)/\{3abc(c^2\alpha_3+c^2\alpha_1)\}$	CB
ζ_c/ζ_o	$2(a^2+b^2)/\{3abc(a^2\alpha_1+b^2\alpha_2)\}$	CC
ρ_a/ρ_o	$2\Big/\left\{\frac{\zeta_o}{\zeta_b}+\frac{\zeta_o}{\zeta_c}\right\}$	RHOA
ρ_b/ρ_o	$2\Big/\left\{\frac{\zeta_o}{\zeta_c}+\frac{\zeta_o}{\zeta_a}\right\}$	RHOB
ρ_c/ρ_o	$2\Big/\left\{\frac{\zeta_o}{\zeta_a}+\frac{\zeta_o}{\zeta_b}\right\}$	RHOC
τ_h/τ_o	$3\Big/\left\{\frac{\rho_o}{\rho_a}+\frac{\rho_o}{\rho_b}+\frac{\rho_o}{\rho_c}\right\}$	TAU
†$\theta_\pm^{red}$	$\frac{abc}{12}\left\{\left(\frac{1}{Q_a}+\frac{1}{Q_b}+\frac{1}{Q_c}\right)\pm\left[\left(\frac{1}{Q_a^2}+\frac{1}{Q_b^2}+\frac{1}{Q_c^2}\right)-\left(\frac{1}{Q_aQ_b}+\frac{1}{Q_bQ_c}+\frac{1}{Q_cQ_a}\right)\right]^{1/2}\right\}$	θ_+^{red}: THETA + or TPLS θ_-^{red}: THETA − or TMNS
‡T_1/T_0	$1/(X_4+X_1)$	T_1
T_2/T_0	$1/(X_4+X_2)$	T_2
T_3/T_0	$1/(X_4+X_3)$	T_3
T_4/T_0	$1/(6X_4-2X_5)$	T_4
T_5/T_0	$1/(6X_4+2X_5)$	T_5
§β	$\frac{N_A^{1/3}}{(16200\pi^2)^{1/3}}\frac{\nu^{1/3}}{f/f_o}$	BETA
R	$\frac{2}{\nu}\left(1+\left[\frac{f}{f_o}\right]^3\right)$	R
δ_i	$\nu\zeta_o/\zeta_i \quad (i = a, b, c)$	DELA, DELB, DELC
γ_i	$(f/f_o)^3\rho_o/\rho_i$	GAMMA A, GAMMA B, GAMMA C
μ_i	$(f_o/f)(\zeta_i/\zeta_o)^{1/3}$	MUA, MUB, MUC

Ψ	$(f/f_o)(\tau_o/\tau_h)^{1/3}$	PSI
Λ	$\nu\tau_o/\tau_h$	LAMBDA
δ_+	$6\nu\theta_+^{red}$	DPLS or DELTA +
δ_-	$6\nu\theta_-^{red}$	DMNS or DELTA −
γ_+	$6(f/f_o)^3\,\theta_+^{red}$	GPLS or GAMMA +
γ_-	$6(f/f_o)^3\theta_-^{red}$	GMNS or GAMMA −

* This term is negligible for globular particles (axial ratios < 3). [8]

† $Q_a = \dfrac{b^2+c^2}{b^2\alpha_2 + c^2\alpha_3}$, $Q_b = \dfrac{c^2+a^2}{c^2\alpha_3 + a^2\alpha_1}$, $Q_c = \dfrac{a^2+b^2}{a^2\alpha_1 + b^2\alpha_2}$.

‡ $X_1 = \dfrac{1}{2}\left\{\dfrac{\rho_o}{\rho_b} + \dfrac{\rho_o}{\rho_c} - \dfrac{\rho_o}{\rho_a}\right\}$, $X_2 = \dfrac{1}{2}\left\{\dfrac{\rho_o}{\rho_c} + \dfrac{\rho_o}{\rho_a} - \dfrac{\rho_o}{\rho_b}\right\}$, $X_3 = \dfrac{1}{2}\left\{\dfrac{\rho_o}{\rho_a} + \dfrac{\rho_o}{\rho_b} - \dfrac{\rho_o}{\rho_c}\right\}$

$X_4 = \dfrac{1}{3}(X_1 + X_2 + X_3)$, $X_5 = \{X_1^2 + X_2^2 + X_3^2 - X_1X_2 - X_2X_3 - X_3X_1\}^{1/2}$.

§ N_A = Avogadro's Number. In the program β is given $\times 10^{-6}$.

3. COMPUTATIONAL METHOD

All the parameters involve an elliptic integral of the form

$$\int_0^\infty f(x)\mathrm{d}x$$

(Appendix). There is at present no packaged or published numerical routine available for integrals of this type; only routines of the form

$$\int_A^B f(x)\,\mathrm{d}x$$

where A can be zero but B must have finite value, specifiable by the user. One such routine used in this program is the Oxford Numerical Algorithms Group's FORTRAN IV NAG D∅1 AGF[3] which uses an internal subdivision strategy developed by Oliver [4] and based on Clenshaw–Curtis quadrature [5].

A satisfactory value for B was determined by using successively higher values until the integral converged to a limiting value. The number of the interval subdivisions (MAXDIV) is also specifiable by the user; the maximum number of fifty was used. The package also estimates the error on the integrals [6]. If this error is greater than the maximum allowable error specifiable by the user the routine will stop and print an error message. The maximum allowable error specified was 1.0×10^{-8} (equivalent to $\sim 0.001\%$). The integrands are specified as an external function (Table 3) and the integrals α_k ($k = 1 \rightarrow 10$) when calculated are stored and called up in the calculation of each shape parameter in the main program, partly shown in Table 2.

4. RESULTS

The results of a sample run for values of (a/b, b/c) of (1.5, 1.5) are given in Table 4. Provided the axial dimensions are known, for example, from X-ray crystallography, the hydrodynamic parameters for any protein can therefore be obtained, provided the general ellipsoid represents a reasonable model for the macromolecular shape, an assumption generally valid for globular proteins. These predicted parameters can be compared with experimentally determined parameters and deductions can be made about whether the crystallographic shape is the same in solution. For example, the crystallographically determined dimensions of myoglobin, a protein of biomedical importance—serving as a reserve supply of oxygen in the muscle tissue of mammals—have been given as $43 \times 35 \times 23$ Å ($a/b = 1.23$, $b/c = 1.52$).

Table 2. Evaluation of the hydrodynamic shape parameters for tri-axial ellipsoids. The WRITE statements at the end of the program have not been included

```
      PROGRAM MAIN(INPUT,OUTPUT,TAPE2=INPUT,TAPE3=OUTPUT)
      COMMON/PARAM/A,C,NN
      EXTERNAL FUN
      DIMENSION ALPHA(10)
      REAL NU,M,O,P
      REAL MUA,MUB,MUC,PSI,N1,N2,RHOA,RHOB,RHOC
C ENTER AS DATA AT THE FOOT OF THE PROGRAM (A/B, B/C)
      READ(2,*)N1,N2
      NN=0
C     COMPUTE THE ELLIPTIC INTEGRALS
      DO45 K=1,10
      A=N1
      B=1.0
      C=1.0/N2
      NN=NN+1
C     SET LIMITS FOR NUMERICAL INTEGRATION (THESE LIMITS
C     BELOW HAVE BEEN PREVIOUSLY TESTED FOR CONVERGENCE)
      AA=0.0
      BB=1000000
      IF(NN.EQ.10)BB=500000000
      MAXDIV=50
      EPS=1.0E-08
      ACC=0.0
      IFAIL=0
C     CALL U.K. "NAG" LIBRARY ROUTINE FOR NUMERICAL EVALUATION
C     OF THE INTEGRALS "ALPHA" GIVEN IN THE SUBROUTINE BELOW
      CALL D01AGF(AA,BB,FUN,MAXDIV,EPS,ACC,ANS,ERROR,NOFUN,IFAIL)
      ALPHA(NN)=ANS
   45 CONTINUE
      B=1.0
C     NOW COMPUTE THE FUNCTION VALUES USING THE STORED INTEGRAL VALUES
      X1=(A*A)-(B*B)
      X2=(C*C)-(A*A)
      X3=(B*B)-(C*C)
      Y1=(A*A)+(B*B)
      Y2=(C*C)+(A*A)
      Y3=(B*B)+(C*C)
      Z1=(A*A*ALPHA(1))+(B*B*ALPHA(2))
      Z2=(C*C*ALPHA(3))+(A*A*ALPHA(1))
      Z3=(B*B*ALPHA(2))+(C*C*ALPHA(3))
      EXT=-(1.0/5.0)*(1.0/(A*B*C))*(((X1/Z1)+(X2/Z2)+(X3/Z3))**2.0)
     +/((Y1/Z1)+(Y2/Z2)+(Y3/Z3))
      NU=(1.0/(A*B*C))*((4.0/15.0)*((ALPHA(7)+ALPHA(8)
     + +ALPHA(9))/(ALPHA(8)*ALPHA(9)+ALPHA(9)*ALPHA
     + (7)+ALPHA(7)*ALPHA(8)))+(1.0/5.0)*(((ALPHA(2)
     + +ALPHA(3))/(ALPHA(4)*(B*B*ALPHA(2)+C*C*ALPHA(3)
     + )))+((ALPHA(3)+ALPHA(1))/(ALPHA(5)*(C*C*ALPHA(3)
     + +A*A*ALPHA(1))))+((ALPHA(1)+ALPHA(2))/(ALPHA(6)
     + *(A*A*ALPHA(1)+B*B*ALPHA(2))))))+EXT
      M=(B*B+C*C)/(B*B*ALPHA(2)+C*C*ALPHA(3))
      O=(C*C+A*A)/(C*C*ALPHA(3)+A*A*ALPHA(1))
      P=(A*A+B*B)/(A*A*ALPHA(1)+B*B*ALPHA(2))
      TPLS=((A*B*C)/(12.0))*((((1.0/M)+(1.0/O)+(1.0/P
     + ))+(((1.0/M**2.0)+(1.0/O**2.0)+(1.0/P**2.0))-
     + ((1.0/(M*O))+(1.0/(O*P))+(1.0/(P*M
     + ))))**0.5))
      TMNS=((A*B*C)/(12.0))*((((1.0/M)+(1.0/O)+(1.0/P
     + ))-(((1.0/M**2.0)+(1.0/O**2.0)+(1.0/P**2.0))-
     + ((1.0/(M*O))+(1.0/(O*P))+(1.0/(P*M
     + ))))**0.5))
      DPLS=6.0*TPLS*NU
      DMNS=6.0*TMNS*NU
      F=2.0/((A*B*C)**(1.0/3.0))*ALPHA(10))
      R=2.0*(1.0+(F**3.0))/NU
      BETA=(1.0/1000000.0)*((6.0249**(1.0/3.0))*(10.0**(23.0/3.0
     +)))/((16200.0*3.141592654*3.141592654)**(1.0/3.0)))*(NU
     +**(1.0/3.0))/F
      CA=(2.0/(3.0*A*B*C))*M
      CB=(2.0/(3.0*A*B*C))*O
      CC=(2.0/(3.0*A*B*C))*P
      RHOA=2.0/((1.0/CB)+(1.0/CC))
      RHOB=2.0/((1.0/CC)+(1.0/CA))
      RHOC=2.0/((1.0/CA)+(1.0/CB))
      DELA=NU/CA
      DELB=NU/CB
      DELC=NU/CC
      TAU=3.0/((1.0/CA)+(1.0/CB)+(1.0/CC))
      PSI=F*((1.0/TAU)**(1.0/3.0))
      V=NU/TAU
      ZZ=(1.0/2.0)*(F**3.0)
      GAMMAA=ZZ*((1.0/CB)+(1.0/CC))
      GAMMAB=ZZ*((1.0/CC)+(1.0/CA))
      GAMMAC=ZZ*((1.0/CA)+(1.0/CB))
      MUA=(CA**(1.0/3.0))/F
      MUB=(CB**(1.0/3.0))/F
      MUC=(CC**(1.0/3.0))/F
      GPLS=6.0*TPLS*(F**3.0)
      GMNS=6.0*TMNS*(F**3.0)
      X1=0.5*((1.0/RHOB)+(1.0/RHOC)-(1.0/RHOA))
      X2=0.5*((1.0/RHOC)+(1.0/RHOA)-(1.0/RHOB))
      X3=0.5*((1.0/RHOA)+(1.0/RHOB)-(1.0/RHOC))
      X4=(X1+X2+X3)/3.0
      X5=((X1**2.0)+(X2**2.0)+(X3**2.0)-(X1*X2)-(X2*X3)-(X3*X1))
     +**0.5
      T1=1.0/(X4+X1)
      T2=1.0/(X4+X2)
      T3=1.0/(X4+X3)
      T4=3.0/((6.0*X4)-(2.0*X5))
      T5=3.0/((6.0*X4)+(2.0*X5))
```

Table 3. A subroutine for specifying the integrands for numerical integration

```
      REAL FUNCTION FUN(X)
      COMMON/PARAM/A,C,NN
      B=1.0
      GOTO(10,20,30,40,50,60,70,80,90,100),NN
   10 FUN=1/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**0.5)
      RETURN
   20 FUN=1/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**0.5)
      RETURN
   30 FUN=1/((A*A+X)**0.5*(B*B+X)**0.5*(C*C+X)**1.5)
      RETURN
   40 FUN=1/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
      RETURN
   50 FUN=1/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
      RETURN
   60 FUN=1/((A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
      RETURN
   70 FUN=X/((A*A+X)**0.5*(B*B+X)**1.5*(C*C+X)**1.5)
      RETURN
   80 FUN=X/((A*A+X)**1.5*(B*B+X)**0.5*(C*C+X)**1.5)
      RETURN
   90 FUN=X/((A*A+X)**1.5*(B*B+X)**1.5*(C*C+X)**0.5)
      RETURN
  100 FUN=1.0/(((A*A+X)*(B*B+X)*(C*C+X))**0.5)
      RETURN
      END
```

Table 4. Sample results for a hypothetical protein of axial ratio $(a/b, b/c) = (1.5, 1.5)$. The results are correct to three decimal places

```
 A/B          1.50    B/C          1.50
 NU       2.8917
  F       1.0441
 THETA+                .1626
 THETA-                .1162
 DELTA+               2.8212
 DELTA-               2.0164
   R            1.4788
 BETA               2.1233
THE 3 ROTATIONAL FRICTIONAL RATIOS:
   CA                 .8976
   CB              1.3980
   CC              1.4704
THE 3 ROTATIONAL RELAXATION TIME RATIOS:
  RHOA             1.4332
  RHOB             1.1147
  RHOC             1.0932
  DELTAA             3.2215
  DELTAB             2.0684
  DELTAC             1.9666
THE HARMONIC MEAN RELAXATION TIME RATIO:
  TAU              1.1954
  PSI               .9837
LAMBDA             2.4188
GAMMAA              .7941
GAMMAB             1.0210
GAMMAC             1.0411
  MUA               .9238
  MUB              1.0709
  MUC              1.0891
GAMMA+             1.1105
GAMMA-              .7937
FLUORESCENCE ANISOTROPY RELAXATION TIME RATIOS:
   T1              1.0253
   T2              1.2888
   T3              1.3187
   T4              1.4340
   T5              1.0249
```

This corresponds to a value of v, using the above alogrithm, of 2.729. That from experiment assuming no swelling is 4.40 ± 0.08 [7], the difference being ascribed either to significant swelling in solution or change of shape.

Prediction of a value for $(a/b,\ b/c)$ from experimentally measured values for the hydrodynamic parameters is much more difficult; this has been discussed in more detail in an earlier study [1].

5. SUMMARY

The theoretical relationships for the hydrodynamic parameters of a general tri-axial ellipsoid in solution in dominant Brownian motion are now available. A general program is presented which, for any given values of the two axial ratios which define a general ellipsoid, calculates the corresponding hydrodynamic parameters. Thus for any ellipsoidal macromolecules whose dimensions are known from crystallographic studies, the corresponding hydrodynamic parameters can be determined.

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APPENDIX

The elliptic integrals

$$\alpha_1 = \int_0^\infty \frac{d\lambda}{(a^2+\lambda)\Delta}; \quad \alpha_2 = \int_0^\infty \frac{d\lambda}{(b^2+\lambda)\Delta}; \quad \alpha_3 = \int_0^\infty \frac{d\lambda}{(c^2+\lambda)\Delta}$$

$$\alpha_4 = \int_0^\infty \frac{d\lambda}{(b^2+\lambda)(c^2+\lambda)\Delta}; \quad \alpha_7 = \int_0^\infty \frac{\lambda d\lambda}{(b^2+\lambda)(c^2+\lambda)\Delta}$$

$$\alpha_5 = \int_0^\infty \frac{d\lambda}{(c^2+\lambda)(a^2+\lambda)\Delta}; \quad \alpha_8 = \int_0^\infty \frac{\lambda d\lambda}{(c^2+\lambda)(a^2+\lambda)\Delta}$$

$$\alpha_6 = \int_0^\infty \frac{d\lambda}{(a^2+\lambda)(b^2+\lambda)\Delta}; \quad \alpha_9 = \int_0^\infty \frac{\lambda d\lambda}{(a^2+\lambda)(b^2+\lambda)\Delta}$$

$$\alpha_{10} = \int_0^\infty \frac{d\lambda}{\Delta} \qquad \text{where } \Delta = [(a^2+\lambda)(b^2+\lambda)(c^2+\lambda)]^{1/2}.$$