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Some Particle Scattering Factors for Rods with Inhomogeneous Mass Distributions. Application to the Molecular Configuration of Myosin[1]

BY ALFRED HOLTZER AND STUART A. RICE[2]

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The angular distribution of scattered light is calculated for a rod consisting of three co-linear sub-units of variable lengths and densities of scattering centers. The general expression obtained is applied to the particular case of the muscle protein myosin, which had previously been shown to be a linear aggregate of three rod-like sub-units (meromyosins), two of which are identical. Within the experimental error the measured distribution is shown to agree with theory only if the two identical units (L-meromyosin) are arranged consecutively in the intact molecule.

I. Introduction

In addition to its utility in determining absolute molecular weights and dimensions of dissolved macromolcules, the light scattering method can also provide information on the shape of the molecular unit. The angular distribution of the scattered light depends markedly on the shape of the molecules and this distribution has been calculated for several uniformly dense geometrical forms.[3-7] The usual method of obtaining shape information therefore consists in comparing the theoretical and observed distributions and choosing that model which best fits the experimental data.

The method clearly rests on the assumptions that: (a) polydispersity of molecular weight and/or shape is absent, or can be accounted for; (b) the correct model is one of those that have been calculated theoretically; *i.e.*, the actual molecule is a rod, random coil, stiff coil, sphere, ellipsoid or cylinder of uniform density.

The effects of polydispersity on the angular scattering envelope have received a great deal of attention and in many cases, if the distribution of weights or sizes can be characterized, the experimental data can still be used in a shape determination.[4,8-12]

Further, the number and variety of geometrical forms for which theoretical envelopes have been calculated would seem to blanket the gamut of possibilities so well that, to the degree of approximation inherent in the measurements, almost any shape molecule could be accommodated. The possibility remains, however, that a given molecule may not have a uniform density of scattering centers. In that case the observed scattering envelope could not be expected to agree with the theoretical curve for the uniformly dense geometrical figure of the same gross shape.

The possibility of the failure of assumption (b) due to non-uniformity in mass distribution was given added weight during the course of a study of the molecular configuration of the muscle protein myosin in solution. All the experimental values of molecular parameters agreed well with the rod model for this molecule.[13] A subsequent analysis of the shape of the angular scattering envelope, however, showed measurable deviations from the theoretical rod-formula at high scattering angles. Since ultracentrifugal studies had shown the presence of only a single molecular species, polydispersity could reasonably be ruled out if not definitely precluded. It is well known that the myosin molecule can be broken up into rod-like subunits of differing molecular weights,[14] consequently it was of interest of investigate theoretically the effect of non-uniform distributions of scattering centers on the scattering envelopes of rod-like molecules, and then to see whether the myosin data could be fitted in this way.

We present first the derivation for a rod with a particular kind of non-uniformity. The application of this calculation to myosin will follow.

II. Theory

We choose as a model a rod with three colinear sub-units of lengths l_1, l_2 and l_3; numbers of scattering centers N_1, N_2 and N_3; and therefore linear densities of scattering centers ρ_1, ρ_2 and ρ_3, respectively (see Fig. 1). The rod thickness is assumed to be negligible compared to the wave length of the light used in scattering experiments.

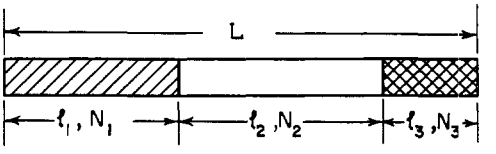

Fig. 1.—Model for rod with inhomogeneous mass distribution. Diameter of rod is assumed to be small compared to wave length of light.

The particle scattering factor, $P(\theta)$, which characterizes the angular dependence of the scattered light due to intramolecular interference is given by the double sum over all pairs of scattering centers, m and n[5]

$$P(\theta) = \frac{1}{N^2} \sum_m \sum_n \frac{\sin \mu r_{mn}}{\mu r_{mn}} \qquad (1)$$

with $N = N_1 + N_2 + N_3$, $\mu = 4\pi/\lambda' \sin(\theta/2)$, λ' being the wave length of light in the solution, and r_{mn} the distance between centers m and n.

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(2) Junior Fellow, Society of Fellows, Harvard University; on leave at Yale University 1956-1957.

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For convenience the sum in (1) may be separated into

$$N^2P(\theta) = \sum_{m=1}^{N_1}\sum_{n=1}^{N_1}\frac{\sin\mu r_{mn}}{\mu r_{mn}} + \sum_{m=1}^{N_2}\sum_{n=1}^{N_2}\frac{\sin\mu r_{mn}}{\mu r_{mn}} + \sum_{m=1}^{N_3}\sum_{n=1}^{N_3}\frac{\sin\mu r_{mn}}{\mu r_{mn}} + \sum_{m=1}^{N_1}\sum_{n=1}^{N_2}\frac{\sin\mu r_{mn}}{\mu r_{mn}} + \sum_{m=1}^{N_2}\sum_{n=1}^{N_3}\frac{\sin\mu r_{mn}}{\mu r_{mn}} + \sum_{m=1}^{N_1}\sum_{n=1}^{N_3}\frac{\sin\mu r_{mn}}{\mu r_{mn}} \quad (2)$$

where the first three terms are related to the particle scattering factors of sub-units 1, 2 and 3 when isolated, and the other three represent the contribution due to interference between pairs of scattering centers located on different sub-units.

Equation 1 may be re-written as

$$\sum_m\sum_n\frac{\sin\mu r_{mn}}{\mu r_{mn}} = \int_0^L F(r)\,\frac{\sin\mu r}{\mu r}\,dr \quad (3)$$

with $F(r)$ the number of scattering centers separated by the distance r. The problem then reduces to finding a suitable analytic form for $F(r)$ for each of the terms in (2).

The first term of (2) is obviously equal to $N_1{}^2P_1(\theta)$ where $P_1(\theta)$ is the well known particle scattering factor for a rod of length l_1. Analogous relations exist for the second and third terms of (2). It remains for us to evaluate the last three terms. Terms four and five involve interference between pairs located on different, but adjacent sub-units. We proceed to evaluate these.

For the fourth term, simple considerations suffice to show that

$$\begin{cases} F(r) = 2\rho_1\rho_2 r & 0 \le r \le l_2 \\ F(r) = 2\rho_1\rho_2 l_2 & l_2 < r \le l_1 \\ F(r) = 2\rho_1\rho_2(l_1 + l_2 - r) & l_1 < r \lesssim L \end{cases} \quad (4)$$

with analogous relations holding for the fifth term.

Similarly, the correlation between pairs on the non-adjacent first and third sub-units lead to the relations

$$\begin{cases} F(r) = 0 & 0 \le r \le l_2 \\ F(r) = 2\rho_1\rho_3(r - l_2) & l_2 < r < (l_2 + l_3) \\ F(r) = \rho_1\rho_3 l_3 & (l_2 + l_3) \le r \le (L - l_3) \\ F(r) = 2\rho_1\rho_3(L - r) & (L - l_3) < r \le L \end{cases} \quad (5)$$

Substitution of the relations (4) and (5) into eq. 3 followed by straight forward integration then leads to the lengthy relation

$$P(\theta) = \left(y_1{}^2 - \frac{\rho_2}{\rho_1}y_1{}^2\right)\left[\frac{1}{x_1}\mathrm{Si}(2x_1) - \left(\frac{\sin x_1}{x_1}\right)^2\right] + \left(y_2{}^2 - \frac{\rho_1}{\rho_2}y_2{}^2 - \frac{\rho_3}{\rho_2}y_2{}^2 + \frac{\rho_3\rho_1}{\rho_2\rho_2}y_2{}^2\right)\left[\frac{1}{x_2}\mathrm{Si}(2x_2) - \left(\frac{\sin x_2}{x_2}\right)^2\right] + \left(y_3{}^2 - \frac{\rho_2}{\rho_3}y_3{}^2\right)\left[\frac{1}{x_3}\mathrm{Si}(2x_3) - \left(\frac{\sin x_3}{x_3}\right)^2\right] + \left(y_1 + \frac{\rho_1}{\rho_2}y_2\right)^2\left(\frac{\rho_2}{\rho_1} - \frac{\rho_3}{\rho_1}\right)\left[\frac{1}{x_4}\mathrm{Si}(2x_4) - \left(\frac{\sin x_4}{x_4}\right)^2\right] + \frac{\rho_3}{\rho_1}\left(y_1 + \frac{\rho_1}{\rho_2}y_2 + \frac{\rho_1}{\rho_3}y_3\right)^2\left[\frac{1}{x_5}\mathrm{Si}(2x_5) - \left(\frac{\sin x_5}{x_5}\right)^2\right] + \left\{\frac{\rho_2}{\rho_3}\left(y_3 + \frac{\rho_3}{\rho_2}y_2\right)^2 - \frac{\rho_3}{\rho_1}\left(\frac{\rho_1}{\rho_2}y_2 + \frac{\rho_1}{\rho_3}y_3\right)^2\right\}\left[\frac{1}{x_6}\mathrm{Si}(2x_6) - \left(\frac{\sin x_6}{x_6}\right)^2\right] \quad (6)$$

where

$$\begin{aligned} x_1 &= \mu l_1/2 \\ x_2 &= \mu l_2/2 \\ x_3 &= \mu l_3/2 \\ x_4 &= \mu(l_1 + l_2)/2 \\ x_5 &= \mu L/2 \\ x_6 &= \mu(l_2 + l_3)/2 \end{aligned} \quad (7)$$

and

$$y_1 = N_1/N;\ y_2 = N_2/N;\ y_3 = N_3/N \quad (8)$$

We note that $P(\theta)$ for this model is the weighted sum of six different particle scattering factors for rods of lengths related to the lengths of the sub-units; where the weighting factors depend on the linear density and fraction of scattering centers in the various units.

Also, as the scattering angle approaches zero the six particle scattering factors in the sum approach unity; and since it can be seen that the sum of their coefficients approaches unity, the total $P(\theta)$ is indeed correctly normalized.

We will now use equation 6 to interpret the observed angular scattering envelope of myosin.

III. The Myosin $P(\theta)$

From a study of the light scattering and hydrodynamic properties of myosin solutions it has been shown that the molecular unit is rod shaped. In particular, if the uniform rod model is accepted sedimentation and viscosity studies coupled with light scattering results show the molecular weight of the molecule to be 530,000, the length 1650 Å. and the diameter of the rod 26 Å.[13] The data definitely rule out the random coil model.

In a series of interesting experiments A. G. Szent-Györgyi has shown that if myosin is digested with trypsin for twelve minutes, it is broken down into two components called meromyosins.[15] These sediment at different rates in the ultracentrifuge, and area measurements show that the faster component (heavy, or H-meromyosin) makes up 57%, and the slower one (light, or L-meromyosin) 43% of the total mass of the myosin. Analysis indicates that there is no material unaccounted for, *i.e.*, the entire mass of the myosin molecule is the sum of the masses of the sub-units produced. From sedimentation and diffusion studies Szent-Györgyi finds that the two different species are each rods with molecular weights 232,000 and 96,000 and lengths of 435 and 549 Å. respectively. The weight percentage figures and these molecular weights reveal an empirical mole ratio of 2L- to one H-meromyosin in the intact myosin. Further, it has recently been shown that the optical rotation of the mero-myosins, properly summed, gives the same value as the measured optical rotation of myosin. This suggests that the sub-units retain the same molecular configuration as in the original molecule.

If this simplest ratio is the correct one, myosin should have a molecular weight of 424,000, which differs by approximately 20% from the value cited above. In view of the cumulative experimental errors in comparing three molecular weights this difference is probably not significant. Further, if the sub-units are arranged end to end the total length of the myosin would be 1533 Å., also in satisfactory agreement with the observed value of 1650 Å. A final check involves the diameters; Szent-Györgyi finds 29 Å. for the H- and 17 for L-meromyosin. These compare favorably with our value of 26 Å. which presumably should be some kind of average.

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Accepting this picture of myosin there are obviously two possible linear arrangements of the sub-units: LLH and LHL. Each of these is characterized by a non-uniform linear mass distribution. Examination of the experimental reciprocal radiation envelope for undigested myosin shows a considerable deviation from the theoretical envelope of a uniform rod of the same radius of gyration (see Fig. 2). Two questions are immediately posed by this observation. First, can we explain the deviation in terms of the effect of non-uniform mass distribution on $P(\theta)$; and, second, can we distinguish between the two arrangements LLH and LHL?

It is most convenient to compare radiation envelopes when they have the same initial slopes. This means that the models considered must be adjusted to have the same radius of gyration, k. The comparison is further complicated by the fact that the relationship between total length and radius of gyration is by no means the same for uniform and non-uniform rods. Some small adjustment of the experimental values is therefore essential to facilitate the comparison. For the sake of clarity we first outline our procedure for calculating $P(\theta)$ for the non-uniform rods and then show the details.

Procedure in calculation of $P(\theta)$ for non-uniform rods: (a) Use the experimental values of l_2/l_1, N_2/N_1 (and therefore ρ_2/ρ_1) to find the center of gravity of the rod. (b) Use results in (a) to obtain the constant factor relating k^2 to L^2. (c) Use same ρ_2/ρ_1 and N_2/N_1 to calculate the coefficients in equation 6. (b) Calculate $P(\theta)$ from (6) using coefficients from (c) and choosing *absolute* lengths so that $\overline{(k^2)}^{1/2} = 475$ Å., the observed value for myosin.

We will now use this recipe to calculate $P(\theta)$ for our two possibilities. We assume throughout the actual calculations that the refractive index increment is the same for all sub-units, so that the number of scattering centers is simply proportional to the mass. Since almost all proteins have values of refractive index increment in the range 0.19–0.2 cc./g., this is not a serious restriction.

LLH Model.—For this model $l_1 = l_2$ and $N_1 = N_2$ (Fig. 1). The relevant ratios are

$$l_3/l_1 + l_2 = \frac{435}{2 \times 549} = 0.400 \qquad (9)$$

$$N_3/N_1 + N_2 = \frac{232{,}000}{2 \times 96{,}000} = 1.21$$

and

$$\frac{\rho_3}{\rho_1} = \frac{\rho_3}{\rho_2} = 3.04$$

With these values we find the center of gravity to be $0.116 \times 2 \times 549$ Å. units to the left of the heavy piece. Using this we find the radius of gyration to be given by

$$k^2 = L^2/11.8 \qquad (10)$$

Using (9) in (6) we find the following expression for $P(\theta)$ for this model

$$P(\theta) = -0.420P_1(\theta) + 0.200P_2(\theta) + 1.22P_3(\theta) \qquad (11)$$

where $P_1(\theta)$, $P_2(\theta)$, and $P_3(\theta)$ are the particle scattering factors for uniform rods of lengths $(l_1 + l_2)$, l_3 and $(l_1 + l_2 + l_3) = L$, respectively.

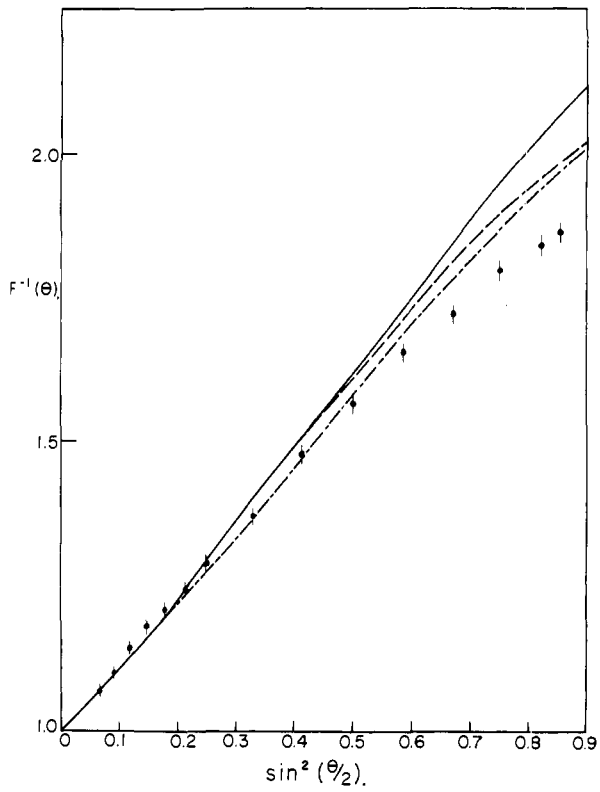

Fig. 2.—Reciprocal particle scattering factors: solid curve, homogeneous, thin rod; dashed curve, LLH model; dotted and dashed curve, LHL model. Experimental points are for intact myosin.

From (10), using the radius of gyration, 475 Å., we find $L = 1630$ Å. For the experimental *ratios* of the sub-unit lengths and this total length we get $l_1 = 582$ Å. and $l_3 = 466$ Å. It is clear that this small adjustment works no great hardship on the experimental values for the meromyosins. Using these last lengths, then, in equation 11 we obtain the dashed curve of Fig. 2.

LHL Model.—In this case we have $l_1 = l_3$ and $N_1 = N_3$ (Fig. 1). Therefore

$$l_2/l_1 = \frac{435}{549} = 0.800$$

$$N_2/N_1 = 2.42 \qquad (12)$$

$$\rho_2/\rho_1 = 3.04$$

and using the obvious center of gravity we find

$$k^2 = L^2/18.2 \qquad (13)$$

Using (12) in (6) we obtain

$$P(\theta) = -0.208P_1(\theta) + 0.134P_2(\theta) + 0.674P_4(\theta) + 0.400P_5(\theta) \qquad (14)$$

where the successive $P_i(\theta)$'s in the right-hand member are those for uniform rods of lengths l_1, l_2, $(l_1 + l_2)$, and L, respectively.

Using our experimental radius of gyration in (13) we obtain a total length of 2030 Å. This means $l_1 = 725$ Å. and $l_2 = 580$ Å. These latter values are some 30% larger than the measured lengths for the meromyosins. The $P(\theta)^{-1}$ for this model is shown as the dot-and-dash curve in Fig. 2.

It is clear from Fig. 2 that while in both these cases the non-uniform density has accounted for

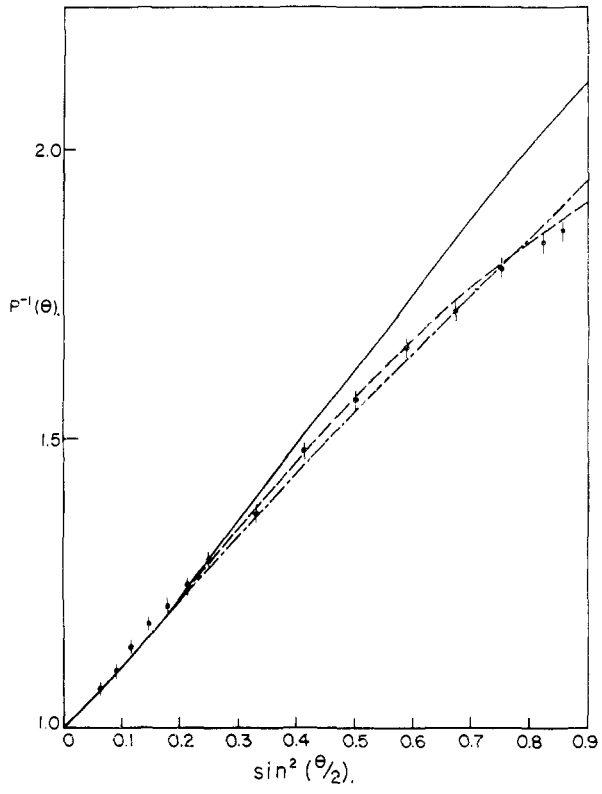

Fig. 3.—Reciprocal particle scattering factors: solid curve, homogeneous, thin rod; dashed curve, adjusted LLH model; dotted and dashed curve, adjusted LHL model. Experimental points are for intact myosin.

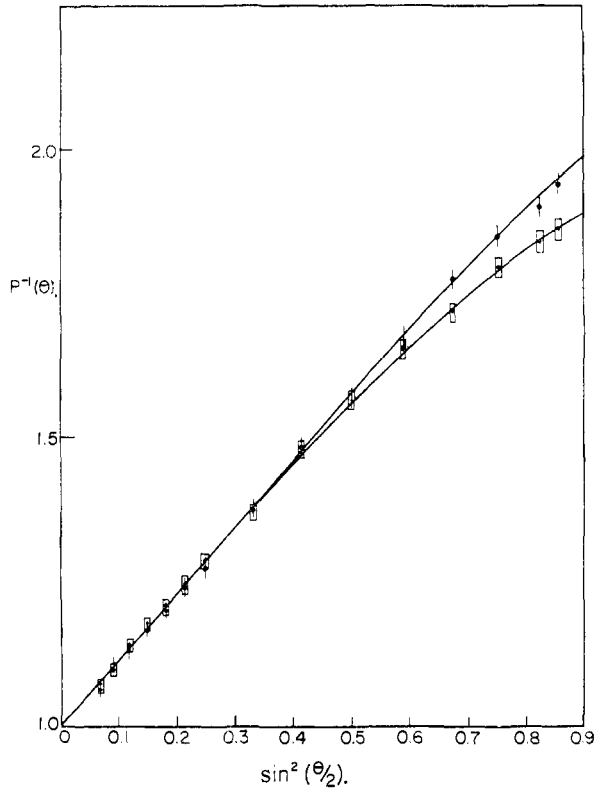

Fig. 4.—Reciprocal particle scattering factors: vertical lines, experimental points for aggregated sample, molecular weight 1.7×10^6; rectangles, experimental points for "monomeric" myosin, molecular weight 530,000.

part of the deviation of experiment from theory, the difference is still significant. We see further that the price we pay for reducing this difference in the LHL case is to make the lengths of the meromyosins too large for comfort.

It should be recalled that the method used by Szent-Györgyi to obtain the meromyosin lengths involved assuming no hydration of the molecules, using the observed fractional coefficient to obtain an axial ratio, and then calculating the length assuming rods of known density. For molecules as small as the meromyosins this procedure is prone to errors even aside from those involved in the arbitrary assumptions about hydration. A reasonable estimate of the error would be of the order of $\pm 20\%$ of each length. The error in their molecular weight values is probably more nearly of the order of $\pm 10\%$.

By adjusting the lengths and masses of the meromyosins within these limits it *is* possible to obtain a theoretical envelope that fits the observed points quite well. Consider the following choices for the two possible models.

LLH Model.—Suppose the correct molecular weight of L-meromyosin is 84,000 and of H-meromyosin 259,000. Suppose further that the lengths are 640 and 387 A. for L- and H-, respectively. Myosin would then have a total mass of 427,000, and a total length of 1665 Å. Since the center of gravity can be shown to be 0.2104×640 Å. to the left of the heavy piece, we get

$$k^2 = L^2/12.25 \qquad (15)$$

from which we see that this length gives us the correct radius of gyration. Using our now familiar procedure we find for this case

$$P(\theta) = -0.636P_1(\theta) + 0.296P_2(\theta) + 1.340P_3(\theta) \qquad (16)$$

where the $P_i(\theta)$'s have the same meaning as in (11).

The reciprocal of this particle scattering factor is shown as the dashed line in Fig. 3. It is seen that theory agrees very well with experiment, and, it is fair to say, all masses and lengths have been chosen within the experimental errors.

We must now consider the LHL case for these same *ratios* of masses and lengths of H- to L-meromyosin.

LHL Model.—For this configuration, with these ratios, we find

$$k^2 = L^2/22.2 \qquad (17)$$

We see that in order to maintain the radius of gyration at 475 Å. the total molecular length would have to be 2240 Å. A proportionate increase in the meromyosin lengths gives us 520 and 860 Å. for H- and L-, respectively. This length for L-meromysin is over 50% greater than the observed length.

Plunging blindly ahead we obtain for this model

$$P(\theta) = -0.318P_1(\theta) + 0.238P_2(\theta) + 0.818P_4(\theta) + 0.262P_5(\theta) \qquad (18)$$

which is plotted as the dot-and-dash curve on Fig. 3. While this curve fits the myosin experimental data almost as well as the dashed curve, the price we pay in terms of disagreement with the mero-

myosin length measurements is too great, particularly since any hydration would make the reported lengths too large.

We may safely conclude that all of the data on both the meromyosins and intact myosin can be explained, within the experimental errors, by the sub-unit arrangement LLH and that the molecule is probably of this form.

There is some further, fragmentary evidence supporting this choice of model. Myosin has been observed to aggregate in a completely side to side fashion.[15] If the LHL arrangement were correct, side to side aggregation would leave the mass distribution and, consequently, $P(\theta)$ unchanged. In the LLH case, however, so long as the cross-linking groups are not all on the heavier unit, one can have dimers of the form $\begin{Bmatrix}\mathrm{LLH}\\ \mathrm{HLL}\end{Bmatrix}$ as well as $\begin{Bmatrix}\mathrm{LLH}\\ \mathrm{LLH}\end{Bmatrix}$. The side to side aggregation would then tend to homogenize the mass distribution and thus to straighten out $P(\theta)^{-1}$. The latter is in fact the case. Scattering envelopes of aggregated samples always show measurably less downward curvature at high angles than monomeric myosin. (See Fig. 4).

It has become increasingly clear in recent years that the derivation of shape information from light scattering data is a hazardous affair. The increasing number of variables known to affect the scattering curve appear to make it more and more difficult to interpret in terms of any model. It should be pointed out, however, that these factors can actually add immeasurably to the strength of the method. If polydispersity has been shown to affect the shape of the curve, it must also be kept in mind that, coupled with the theory, that same curve can now serve to inform us on the nature of the polydispersity. The same could be said of the effect of inhomogeneous mass distributions, as we have tried to show here in a particular case.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA AND THE INSTITUTE OF MOLECULAR PHYSICS OF THE UNIVERSITY OF MARYLAND]

A Simple Model for Barriers to Internal Rotation. II. Rotational Isomers[1]

BY MAURICE M. KREEVOY AND EDWARD A. MASON

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The energy differences between rotational isomers in substituted ethanes and between *cis–trans* isomers in substituted ethylenes have been calculated from a model which assumes spherical symmetry for atoms in molecules. Force laws are taken by analogy with known forces between similar atoms or groups of atoms which are not a part of a larger molecule, with allowances made for any residual electrical charges. No disposable parameters are involved in the calculations. Good agreement is obtained for the substituted ethanes. For the substituted ethylenes the *cis* form is always found to be more stable than calculated, and the difference is attributed to resonance involving a partial double bond between the substituent and the ethylenic carbon.

Introduction

A simple model for interactions between nonbonded atoms or groups of atoms has recently been applied to the calculation of symmetrical barriers to internal rotation about single bonds.[2] The model is now extended to the calculation of energy differences between *trans* and *gauche* forms of substituted ethanes, and energy differences between *cis* and *trans* disubstituted ethylenes. For the substituted ethanes the agreement with experiment is quite good and it is felt that these energy differences are now substantially understood. To explain the results for the dihaloethylenes something more than steric and electrostatic effects is required. A resonance effect such as that proposed by Pitzer and Hollenberg[3] seems likely. No disposable parameters are involved in any of the present calculations.

The Model.—The present simplified model estimates the van der Waals repulsions between nonbonded atoms or groups of atoms in molecules by analogy with known repulsions between atoms or groups of atoms which are not part of a larger molecule. For example: the interactions between two fluorine atoms bonded to different parts of a larger molecule are assumed to be the same as the interactions between two isolated neon atoms; the interactions between two non-bonded chlorine atoms the same as those between two isolated argon atoms. The force laws describing these interactions are taken from well-founded quantum mechanical calculations or from experimental results, so that no disposable parameters are involved in the procedure.

The force laws to be used in the present calculations already have been given,[2] with the exception of the bromine and the iodine interactions, which are taken by analogy with krypton and xenon, respectively. Unfortunately, the potential functions for these systems are not as well known experimentally as for the other systems. From a number of potential functions proposed for interactions between krypton atoms,[4,5] we have selected the following as a suitable representative

$$\varphi(r) = (4.694 \times 10^4) \exp(-2.76r) - 3888/r^6 \text{ kcal./mole} \quad (1)$$

where r is the separation distance in Å. This potential function was derived from measurements on second virial coefficients at high temperatures,[6]

(1) This research was supported in part by Contract Nonr 595(02) of the U. S. Office of Naval Research at the University of Maryland.

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