MOLECULAR SIZE, SHAPE AND AGGREGATION IN CONCENTRATED PROTEIN SOLUTIONS AS REVEALED BY X-RAY SCATTERING

HAEMOGLOBIN AND EGG-ALBUMIN

by

D. P. RILEY

The Royal Institution, London, W. 1 (England)

AND

D. HERBERT

Medical Research Council Unit for Bacterial Chemistry, The Lister Institute, London, S.W. 1 (England)

In the course of a systematic study of the scattering of X-rays by protein solutions, we have obtained results with highly concentrated solutions of human haemoglobin and of egg-albumin which, although preliminary in nature, show that it is possible to derive in a quasidirect manner the dimensions of the hydrated protein molecule. This constitutes a new technique for the determination of macromolecular weight and shape, although further experimental and theoretical refinements are necessary to render the method more exact. It would seem that this fresh application of the technique of low-angle scattering of X-rays may be developed into a useful tool for the study of colloidal and biological systems.

PREPARATION OF SOLUTIONS

The egg-albumin used in this work was prepared from egg-white by half-saturation with $(NH_4)_2SO_4$, and crystallization at p_H 4.6; it was 3 times recrystallized by the same method. Haemoglobin from washed human red cells was crystallized from $(NH_4)_2SO_4$ by the method of DRABKIN[1].

The preparation of highly concentrated solutions of these proteins is not an altogether simple matter. Fairly concentrated solutions could be prepared by filtering the crystals (with suction and thorough draining) and direct solution in the minimum amount of water. The maximum concentrations obtainable in this way are limited by the $(NH_4)_2SO_4$ in the mother liquor adherent to the crystals; more concentrated solutions were obtained as follows.

Protein solutions obtained as above were dialysed against distilled water until salt-free, and dried from the frozen state at $-40°$ C in a centrifugal "spin-freeze" drier. Saturated solutions were prepared from the dried proteins by adding rather less than an equal weight of distilled water, and allowing to stand with occasional stirring for 24 hours at 0°; undissolved protein was removed by centrifugation for 1 hour at 0° in a high-speed angle centrifuge (20000 g).

Contact of the dried haemoglobin with air was minimized, since it leads to methaemoglobin formation (cf. DRABKIN, *loc. cit.*). The solutions contained not more than 3% methaemoglobin, and gave a negative haemochromogen test, showing absence of any denatured protein. Solutions prepared directly from the crystals contained no methaemoglobin.

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Except when actually being photographed, all specimens were kept sealed in a refrigerator.

The weight/volume concentrations of the most concentrated solutions obtained were: Haemoglobin 57.9%, egg-albumin 42.4%.

THE X-RAY PHOTOGRAPHS

The X-ray photographs were taken with nickel-filtered CuK α radiation, $\lambda = 1.54$ Å, in a vacuum camera with specimen-to-film distances of 15 to 40 cm. The 50 kW-rotating-target tube in the Davy-Faraday laboratory enabled us to obtain sufficiently intense photographs with exposure times of one to five hours. In general, the solutions were sealed off carefully in thin walled (0.02–0.03 mm). Perspex or Hysil glass tubes of about 1 mm bore. The 58% haemoglobin solution, however, was far too viscous to allow this and was photographed in a small cell made of polythene by Dr. J. B. FINEAN.

As described in more detail in the next section, these concentrated solutions give X-ray photographs which show one or more bands of a diffuse nature. It is obvious that such maxima in the angular distribution of scattered X-ray intensity arise from the nearness of approach of neighbouring molecules in strong solutions. At low diffraction angles, each molecule may be considered as a unit of homogeneous scattering power, this power being equal to the appropriate electron density for the protein less that for the solvent (water or salt solution*). The systems are therefore similar, from the standpoint of diffraction theory, to monatomic liquids, but with one or two differences. First, the molecules may not be spherical whereas atoms are; secondly, their zwitterion structures and large mass may allow a more pronounced local ordering than in the case of single atoms, where the thermal agitation is relatively more active.

The most rigorous approach to the interpretation of the diffraction effects would be in a FOURIER analysis of the whole course of the intensity/angle curve. Experience with other systems[2, 3], however, has shown that significant data may be derived by considering the positions of the *maxima* only. It is convenient for this purpose to specify the *Bragg spacing* of a band:

$$d_B = \frac{\lambda}{2 \operatorname{Sin} \Theta} \approx \frac{\lambda}{\operatorname{Sin} \Phi}$$

where Φ is the diffraction angle corresponding to the peak of the band.

The Bragg spacing is a significant quantity only when the repetition of scattering units is sufficiently perfect and sufficiently sustained. There must be long-range order as in macroscopic crystals, and the existence of such perfection is demonstrated by the number and sharpness of the diffraction lines given by such a specimen. The Bragg spacings here are related in a simple geometrical way to the separation of neighbouring repeat-units.

In solutions, however, the molecular ordering is, in general, less regular and the mean centre-to-centre distance of neighbouring molecules is given by

$$s = K d_B$$

The numerical factor K is a somewhat arbitrary quantity which must be expected to vary according to circumstances. In the case of close-packed spherical micelles, SCHULMAN AND RILEY[3] have shown that, with K = 1.23, good agreement is obtained with dimensions[4] derived by the independent method of light scattering. This value

* The fact that salt-free solutions were used in mose cases means that only water can lie between the protein molecules and this simplifies interpretation.

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has been suggested for close packed monatomic liquids (see RANDALL[5]) and also for lamellar soap systems where the number of repeating layers is small[6]. It can be shown, in the case of close-packed spheres, to be related to the geometry of the array. For hexagonal or cubic close-packing (12-coordination), or for body-centred packing (8-coordination), the centre-to-centre distance separating nearest neighbours

$$s = \sqrt{\frac{3}{2}}\, d_B = 1.23\, d_B$$

where d_B = the spacing of the following planes in the crystalline state:

0002	(hexagonal close-packing)
111	(cubic close-packing)
110	(body-centred packing)

This factor might reasonably be expected to apply in the case of concentrated macromolecular solutions, if the molecules are near-spherical. A similar geometric argument leads to the relation

$$s = \frac{2}{\sqrt{3}}\, d_B = 1.15\, d_B$$

for the separation along their diameters of close packed cylinders (6-coordination), where d_B is the spacing of the 100 planes in the crystalline state, or of the 10 planes in the nematic paracrystalline state.

The fundamental assumption underlying the interpretations put forward in this paper is that, in these exceedingly concentrated solutions, there is a pseudo-lattice structure. That is to say, a semi-crystalline arrangement of molecules is pre-supposed, the regularity of which is sufficient to allow of simple geometric reasoning without risk of serious error. A forthcoming publication (by G. OSTER AND D. P. RILEY) will attack the theoretical problem from a different standpoint—that of radial distribution functions—and no assumptions regarding the degree of crystallinity will be made. The present paper, however, is consistent in its approach within the limits of the underlying assumption and examines the problem on the simplest theoretical basis.

RESULTS

Haemoglobin

The 58 % solution gave two clearly resolved diffraction bands (Fig. 1) of Bragg spacings 54.5 and 32.5 Å. The latter is much the weaker band. Without other data, it is difficult to proceed to an unequivocal derivation of the molecular dimensions. If, however, we compare these values with PERUTZ's well-established picture of the haemoglobin molecule (of horse)[7] as a squat cylinder of height 34 Å and diameter 57 Å, we can go a stage further. Assuming a monomolecular layer of water of hydration 3 Å thick, PERUTZ's molecules, when hydrated, would be 40 Å high and have a diameter of 63 Å. It will be seen that these figures agree exactly with those derived from the Bragg spacings for the solution, if the appropriate correction factors are used.

Thus $s_1 = Kd_B = 1.15 \cdot 54.5 = 63$ Å (K = 1.15 for cylinder diameters)

and $s_2 = Kd_B = 1.23 \cdot 32.5$ Å (K = 1.23 for cylinder heights)

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We have, admittedly, proceeded in an empirical fashion and used PERUTZ's molecular dimensions for horse haemoglobin in order to be guided in the choice of correction factors. Nevertheless, the choice is reasonable. In a system composed of close-packed cylinders but where short range order only exists, one would *a priori* assume different factors to apply to the separation along diameters or heights of the cylinders. The geometrical factor $\frac{2}{\sqrt{3}} = 1.15$ applies to the diameters and the factor 1.23 has reference to the limited number of repeats along the heights.

On the basis of this picture, the dimensions of the haemoglobin molecule are identical in man and horse and, in sufficiently concentrated solution, the hydrated molecules aggregate in a close-packed and partially ordered fashion. That the molecules probably aggregate into *micelles* separated by the surplus water is shown by comparing the known volume/volume concentration with the volume/volume ratio in a close-packed system, thus

volume of water/volume of protein = 1.30 (58% wt/vol. solution)

volume of water/volume of protein = 0.58 (close packed hydrated cylindrical molecules)

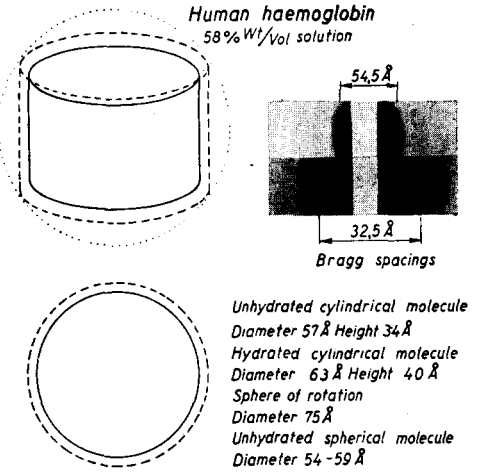

Fig. 1. X-ray photograph of saturated human haemoglobin solution. Spacings given are Bragg spacings and, where used, the appropriate correction factors are given in the text. Possible molecular models shown on left. Unhydrated molecules indicated with bold lines and hydrated molecules with dashes; sphere of rotation of hydrated cylindrical molecule with dots. The lower photograph has been heavily printed to show up the faint outer band, while the less dense upper print shows the inner band clearly resolved.

The more dilute solutions show only one diffraction band. A 25.6 % wt/vol. salt-free solution gives a broad and not well resolved band of approximate spacing, $d_B = 77$ Å, whereas a 36.3 % solution containing about 10 % $(NH_4)_2SO_4$ produces a better resolved band with $d_B \approx 64$ Å. It is evident that in these solutions the cylindrical molecules are free to rotate and that what is being measured is the mean distance of approach of neighbouring spheres of rotation. As there is no longer any local ordering in the sense of preferred orientation of the anisodimensional molecules, only one interference maximum is produced. Using the appropriate factor, K = 1.23, the following centre-to-centre distances between spheres of rotation are arrived at; and the effect of dilution is clearly illustrated:

36.3 % solution, s = 79 Å
25.6 % solution, s = 95 Å

PERUTZ[8] has discussed from this point of view the results obtained by DERVICHIAN, FOURNET, AND GUINIER[9] with red blood cells (of horse). They observed a shoulder in the scattering curve which corresponded to $d_B \approx 62$ Å. PERUTZ shows that the equivalent intermolecular distance, s = 75 Å, agrees closely with the diameter of the sphere of rotation of a monomolecularly hydrated molecule. He concludes that the concentra-

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tion of haemoglobin in red cells (34%) is just that which permits free rotation of the hydrated molecule but no more. In our 36% solution, which corresponds roughly to the red cell, a somewhat larger value for the intermolecular distance is found, probably due to the high salt concentration.

An alternative interpretation of our data in terms of an imperfect lattice model can be advanced if the molecules are assumed to be spherical and closely packed*. In this case, the outer band is taken as being equivalent to the $11\bar{2}0$ reflection of a crystalline hexagonally close-packed system, and the inner to an amalgamation of the $10\bar{1}0$ and 0002 spectra. In the crystal the following ratios between these interplanar spacings obtain

$$d_{11\bar{2}0} : d_{10\bar{1}0} = 1.732, \text{ and } d_{11\bar{2}0} : d_{0002} = 1.633$$

whereas the Bragg spacings of the two haemoglobin bands are in the ratio 1.69:1. It follows that the diameter of the molecule (assumed hydrated) is simply twice 32.5 = 65 Å. Once again, if it is assumed that the shell of water of hydration is 3 Å thick, the diameter of the unhydrated spherical molecule is 59 Å approximately, whereas that corresponding to a molecular weight of 66700 is 54 Å. The discrepancy lies within the limits of experimental error. If this interpretation is the correct one, the molecule of human haemoglobin differs appreciably in shape, but not in weight, from that of horse haemoglobin. In this system, the ratio

$$\text{vol. of water/vol. of protein} = 0.81$$

and aggregation into micelles is again indicated. The effect of dilution in this case is to make the system more disordered and to increase the average intermolecular distance.

At this stage, and from our data alone, it is not possible to decide between the two alternative models for the human haemoglobin molecule. A decision might, however, prove possible from a detailed analysis of intensity data obtained with crystal-reflected monochromatic X-rays and this will be attempted in due course.

GUINIER AND FOURNET[10a] also report results on dilute solutions of haemoglobin (5 to 15 %**). These all give a "gas-type" of low-angle scattering which allows the derivation of the radius of gyration only of the molecule, namely 23 Å. GUINIER points out that this value agrees exactly with the radius of gyration of a cylinder of height 34 Å and diameter 57 Å, the unhydrated molecule of PERUTZ.

One more experiment with haemoglobin was performed in an attempt to observe the splitting of the molecule which is reported to occur in strong solutions of urea. To the 25.6 % solution was added solid urea to a concentration of 4 M; the final haemoglobin concentration was then 19.2 % w/v. The X-ray photograph given by this specimen was quite different from that given by the original protein solution and consisted of a general

* We are indebted to Dr D. M. CROWFOOT for this suggestion.

** The low-angle scattering of X-rays by *dilute* protein solutions has been studied in a preliminary fashion by these and other workers. In general, this approach can only lead to an estimation of the *radius of gyration* of the molecule and neither the actual dimensions nor the volume are directly given (see, however, GUINIER[10c], ROESS AND SHULL[19], and KRATKY AND POROD[11c] for various approaches to the theory of scattering by non-spherical particles). Both GUINIER[10b] and KRATKY[11] report that haemocyanin probably associates in dilute solution (*ca* 4%); as in this case, a maximum is observed in the intensity/angle curve. KRATKY also surmises on less clear evidence that dilute insulin solutions exhibit molecular association.

The essence of our method is the use of extremely concentrated solutions, or alternatively, the encouragement of molecular aggregation in more dilute solutions by a suitable choice of electrochemical factors.

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diffusion of the "gas-type". In view of the high concentration of urea present, it is impossible to interpret this result precisely, but it is not inconsistent with a profound alteration in the haemoglobin molecule.

Egg-albumin

The saturated salt-free solution of egg-albumin (42.4 % wt/vol.) shows three separate diffraction effects (Fig. 2).

a) diffuse central scattering in the immediate neighbourhood of the direct beam,

b) a well resolved and not very diffuse band of d_B = 23 Å,

c) a very diffuse band of mean spacing 11.5 Å which is probably the coalescence of two adjacent bands of Bragg spacings 13 and 10.5 Å.

The above order of decreasing spacing is also that of decreasing intensity.

The central scattering appears to be of a plateau type in which the shoulder corresponds to a Bragg spacing of about 60 Å.

Photographs were also taken of 25.4, 17.0 and 8.5 % solutions, *i.e.*, 60, 40 and 20 % saturated. The same general effects were noticed, except that with the two most dilute solutions the 11.5 Å band was not visible. The most striking feature in the series of four photographs is the persistence of the 23 Å band, which is still present, although very weak, in the 8½ % solution.

The spacing of this band remains unchanged within the limits of experimental and observational error, notwithstanding an apparent slight increase with dilution. Any change in spacing, however, is obviously a second order effect and cannot exceed 2 Å over the whole range. The central scattering, on the other hand, moves in markedly with increasing dilution so that the spacing corresponding to the shoulder increases.

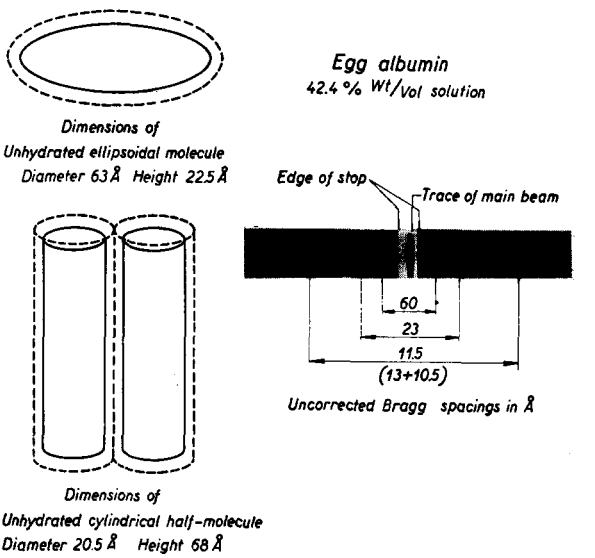

Fig. 2. X-ray photograph of saturated egg-albumin solution. Spacings given are uncorrected Bragg spacings and can refer to either of the molecular models shown on left, the appropriate correction factors being indicated in the text.

CONCENTRATION PER CENT	APPROXIMATE SHOULDER SPACING, d_B
42.4	60 Å
25.4	70 ,,
17.0	85 ,,
8.5	105 ,,

We have now to interpret the various diffraction effects and, of these, the scattering, certainly, and the 23 Å band, possibly, refer to intermolecular distances.

One explanation of the latter band is that the molecules form aggregates and thas this form of association persists even in relatively dilute solutions. This aggregation it

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directional and takes place along the 23 Å direction only. The increase in the 60 Å spacing on dilution shows that the molecules are here simply moving apart, while the notable diffuseness of the scattering suggests a considerable lack of order in this direction.

It is of interest to note that BIER AND NORD[12] have arrived at similar conclusions as a result of light-scattering studies on egg-albumin solutions at the iso-electric point. They show that an appreciable proportion of molecules, even in dilute solutions (1 to 6 %) take part in aggregation and that the effect is much enhanced at higher temperatures.

To derive from the X-ray data alone an unequivocal picture of the unhydrated protein molecule is impossible, yet it is feasible to limit the alternatives if account is taken of the other physical-chemical work on egg-albumin. The molecular weight of this protein is still a subject for discussion, as a number of different values ranging from 34000 to 46000 have been reported. COHN AND EDSALL[13] have critically reviewed the situation and conclude that the physical-chemical evidence points to a value of 43000 ± 3000, the earlier lower values being presumably suspect. BIER AND NORD, on extrapolating their results to infinite dilution, arrive at a molecular weight of 37000, a value much more in accordance with those for other proteins in this class (see RILEY[14]). It will readily be appreciated that the effect of aggregation would be to raise the apparent molecular weight as determined by most physical-chemical methods, and the indefinite amount of such aggregation might well cause considerable differences even in the apparent weights. It seems to us that the values 40–46000 may, for this reason too, be high and that a truer figure is theoretically more likely to be given by the light scattering method, although the molecule is rather too small for accurate measurements. Be that as it may, any molecular model based on our X-ray results must correspond to a molecular weight between the wide limits 34000 to 46000.

We shall start by assuming, by analogy with haemoglobin, that in the saturated solution the intermolecular distances are those between truly hydrated molecules. If the true water of hydration is a monomolecular skin of thickness 3 Å over the whole surface of the molecule, one would need to subtract 6 Å from the intermolecular distances in order to derive the dimensions of the unhydrated molecule. This procedure has been adopted throughout the following argument.

The first model one is tempted to adopt is a cylindrical one, in which case the aggregation might be of the same type as in the saturated haemoglobin solution and the same correction factors could be used. For a prolate cylinder, the intermolecular distances would be 26.5 Å along the diameter and (less exactly) 74 Å along the heights, which would give dimensions of 20.5·68 Å to the unhydrated molecule. These would correspond* to a molecular weight of only 18000. Similar calculations for an oblate cylinder lead to dimensions of 63·22.5 Å and a weight of 56500 for the unhydrated molecule. Incidentally, a square prism still has a molecular weight of only 27500, even though the larger correction factor, 1.23, be used for both dimensions (height 68 Å, edge of square 22.5 Å). It seems, therefore, that none of these molecules has a weight within the very wide range allowed for egg albumin and, at first sight, all of them can be excluded from the list of possibilities. Unfortunately, the matter is not quite so simple for two reasons. First, the long spacing of *ca* 60 Å refers to a shoulder and not to a definite maximum and the real value may be higher than this; second, the correction

* The partial specific volume was taken as 0.749, from the determinations of ADAIR AND ADAIR[15]; according to these authors, it does not vary with the protein concentration.

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factors, K, are not precisely known quantities. A square prism of height 86 Å would have a molecular weight of 35000 and this height would just be permitted by the X-ray data. Similarly, the dimensions of the oblate cylinder could be reduced by using smaller K factors, and the molecular weight thus sufficiently diminished to bring it within the permitted range. Finally, it may be that the "molecule" is really composed of two cylindrical half-molecules of weight 18000 in tight association side-by-side*, in which case the 23 Å band can be considered as intramolecular.

An ellipsoidal model, on the other hand, immediately gives a plausible value for the molecular weight. An oblate ellipsoid of height 22.5 Å and diameter 63 Å would correspond to a molecular weight of 37500, and the dimensions are those derived by using the most probable K factors, namely 1.23 for the height and 1.15 for the diameter. The weight would be increased to 43500 by using K = 1.23 in both directions, in which case the diameter would become 68 Å.

While it is clearly impossible to make a definite affirmation, the balance of evidence leads to two alternatives: a) a flattened ellipsoid of molecular weight 40000 ± 3000, of height *ca* 23 Å and diameter 63–68 Å, the axial ratio a/b ≈ 2.9 is quite a reasonable one, judging by values given by other physico-chemical methods and allowing for the effect of hydration; b) a bi-cylindrical molecule, each cylinder being of height 68 Å and diameter 20.5 Å.

The 11.5 Å band is almost certainly intramolecular in origin, but, owing to its complex nature, it is conceivable that the 13 Å component might correspond to a third intermolecular distance. That this is very probably not the case is shown by the fact that the molecular weights of the corresponding tri-axial ellipsoid or ellipsoidal cylinders are all too small. The 10.5 Å band is obviously related to the prominent interatomic vectors of the same length mapped out in PATTERSON projections and sections of various crystalline proteins. Although this fundamental spacing has long been known from the pioneering work of ASTBURY to exist in fibrous proteins, it is of some interest that it has now been observed in solutions of a globular protein.

COMPARISON WITH OTHER METHODS

A complete physical picture of a protein molecule involves a knowledge of its geometric form, linear dimensions, molecular weight, and degree of hydration. It is of interest to compare the type of information on these points that is given by the X-ray method, as compared with other well-established methods such as osmotic pressure, sedimentation, and diffusion measurements.

Osmotic pressure measurements give a direct estimate of the number of particles in a protein solution, and hence of the mean particle mass; this can be equated to the molecular weight if it is known that the protein is homogeneous and the particles are single molecules. The method gives no information on the shape, size or degree of hydration of the molecules.

Measurements of the sedimentation and diffusion constants allow calculation of

* It is perhaps relevant to this hypothesis that LONGSWORTH, CANNON, AND MACINNES[16] report that several times recrystallized egg albumin has two components which have different electrophoretic mobilities at most p_H values. The fact that the main component has the greater mobility might be held to suggest a dissociation to half-molecules with the equilibrium lying well towards the whole molecule. More recently[17] three electrophoretic constituents have been reported, the third and least mobile being present in only small amount.

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the molecular weight and the ratio, f/f_0, of the observed frictional constant to that of a spherical unhydrated molecule of the same mass. If the hydration can be obtained independently (which is seldom the case), and a particular shape is assumed, then the axial ratios can be calculated from the value of f/f_0. The data themselves give no information on the shape of the molecules, which in practice are always assumed to be prolate or oblate ellipsoids (*e.g.*, ONCLEY[18]).

To summarize, the above methods provide fairly direct estimates of the molecular weight of a protein, but only very indirect estimates, involving several arbitrary assumptions, of the shape and size of the molecule.

The type of information provided by the X-ray data is quite different. This method has the possibility of giving a rather direct estimate of the actual linear dimensions (and not merely the axial ratios) of the hydrated protein molecule. From these, the dimensions of the unhydrated molecule may be obtained if the degree of hydration is known. In the case of haemoglobin, previous data combined with those presented here allow the fairly confident postulation of a monomolecular layer of water of hydration 3 Å thick. In the case of egg-albumin, the degree of hydration is rather less certain, but small errors in the assumed value will not greatly affect the overall picture of the molecule.

The X-ray data do not give the shape of the molecule directly but if an approximate value for the molecular weight is available the allowable shapes are determined.

It is evident from this comparison that the X-ray method is complementary to other physical methods for the study of protein molecules, and provides information on just those points where other methods are deficient. Its main value, we believe, will lie in giving direct information on the size and shape of protein molecules, and on the at present very uncertain question of the hydration of proteins in solution.

Acknowledgements

Dr. J. B. FINEAN kindly took a critical photograph for us during a period when the 50 kW-X-ray tube was out of action, in spite of the efforts of Mr. H. SMITH and Mr. W. A. COATES in maintaining and operating it.

We have also greatly benefited from several stimulating discussions with Prof. E. K. RIDEAL.

SUMMARY

A saturated (57.9% wt/vol.) salt-free solution of human haemoglobin gives two X-ray diffraction bands of Bragg spacings 54.5 Å and 32.5 Å. Two interpretations are possible in terms of close-packed hydrated molecules, each surrounded by a single water layer of 3 Å thickness. The unhydrated protein molecule may be a squat cylinder of height 34 Å and diameter 57 Å, in perfect agreement with the results of PERUTZ with single cystals of horse haemoglobin; alternatively, it may be a sphere of diameter 54–59 Å approximately. On dilution, only one diffraction band is given, the spacing of which increases with decreasing concentration of protein. This could be due either to the free rotation of the cylindrical molecules or to the destruction of an imperfect lattice structure in the case of the spherical molecules.

A saturated (42.4% wt/vol.) salt-free solution of egg-albumin gives three diffraction effects: a central scattering, a band of Bragg spacing 23 Å, and a double band of mean spacing 11.5 Å. The latter is taken to correspond to two prominent intramolecular vectors of length 10.5 Å and 13 Å. The medium spacing band persists on dilution and is possibly due to molecular association or aggregation although this may be between half-molecules. The unhydrated molecular dimensions are uncertain but the most plausible alternatives are:

a) an oblate ellipsoid of height 22.5 Å and diameter 63 Å (molecular weight of 37500);

b) two cylindrical half-molecules of weight 18000 in tight association side-by-side. The dimensions of each cylinder are: height, 68 Å, diameter 20.5 Å.

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RÉSUMÉ

Une solution saturée d'hémoglobine d'homme (57.9% en poids/volume) ne contenant pas de sels donne lieu à deux bandes de diffraction de rayons-X qui correspondent à des équidistances de Bragg de 54.5 Å et 32.5 Å. Si le système est composé de molécules hydratées en assemblage compact, chaque molécule étant entourée d'une couche monomoléculaire d'eau d'une épaisseur de 3 Å, deux interprétations sont possibles. La molécule non-hydratée d'hémoglobine peut être un cylindre aplati de 34 Å de hauteur et de 67 Å de diamètre, laquelle conclusion s'accorde parfaitement avec les résultats de PERUTZ sur des monocristaux d'hémoglobine de cheval, ou elle peut être une sphère de 54 à 59 Å environ de diamètre. Après dilution, une seule bande se produit dont l'équidistance augmente lorsque la concentration de protéine diminue. Ce phénomène peut s'expliquer par le fait que les molécules cylindriques peuvent tourner sur elles-mêmes ou, dans le cas des sphères, à la destruction d'une maille crystalline imparfaite.

Une solution saturée d'ovalbumine (42.4% poids/volume) ne contenant pas de sels produit trois phénomènes de diffraction: une diffusion centrale, une bande d'équidistance (Bragg) de 23 Å, et une bande double d'équidistance moyenne de 11.5 Å. Celle-ci est supposée correspondre à deux vecteurs importants intramoléculaires de 10.5 Å et 13 Å de longueur. La bande de 23 Å persiste après dilution, étant peut-être due à une association ou une agrégation de molécules, bien que celle-ci puisse être entre demi-molécules. Les dimensions de la molécule non-hydratée sont incertaines, mais les formes les plus probables sont:

a) un ellipsoïde aplati de 22.5 Å de hauteur et de 63 Å de diamètre (poids moléculaire 37500);

b) deux demi-molécules cylindriques de poids 18000 liées étroitement côte à côte, les dimensions de chaque cylindre étant: hauteur, 68 Å, diamètre, 20.5 Å.

ZUSAMMENFASSUNG

Eine gesättigte (57.9 Gew. %/Vol.) salzfreie Lösung von menschlichem Hämoglobin gibt zwei X-Strahlen-Beugungsbanden, welche zwischenmolekularen Bragg'schen Abständen von 54.5 Å und 32.5 Å entsprechen. Wenn das System aus enggepackten, wasserhaltigen Molekeln besteht, deren jede von einer 3 Å dicken Einzelschicht von Wasser umgeben ist, so sind zwei Auslegungen möglich. Die wasserfreie Hämoglobinmolekel kann entweder ein flacher Zylinder von 34 Å Höhe und 57 Å Durchmesser sein, in vollkommener Übereinstimmung mit den Ergebnissen von PERUTZ mit einfachen Kristallen von Pferd-Hämoglobin, oder eine Kugel von *ca* 54–59 Durchmesser.

Wird die Lösung verdünnt, so erscheint nur eine Bande deren Abstand zunimmt mit abnehmender Konzentration des Proteins. Dies könnte verursacht werden entweder durch die freie Rotation der zylindrischen Molekeln, oder durch Vernichtung einer unvollkommenen Gitter-Struktur bei sphärischen Molekeln.

Eine gesättigte (42.4 Gew. %/Vol.) salzfreie Lösung von Eieralbumin gibt drei Beugungs-Effekte: eine Zentralstreuung, eine Bragg'sche Bande von 23 Å Abstand und eine doppelte Bande deren mittlerer Abstand 11.5 Å beträgt. Es wird angenommen, dass letztere zwei wichtigen intramolekularen Vektoren von 10.5 Å und 13 Å Länge entspricht. Die Bande mittleren Abstandes (23 Å) bleibt bei Verdünnung bestehen und kann möglicherweise durch molekulare Vereinigungen oder Aggregate hervorgerufen werden. Die Dimensionen der wasserfreien Molekel sind ungewiss, aber die beiden wahrscheinlichsten Formen sind:

a) ein flaches Ellipsoïd von 22.5 Å Höhe und 63 Å Durchmesser (Molekulargewicht: 37500);

b) zwei zylindrische Halbmolekeln (Molekulargewicht je 18000) in enger seitlicher Verbindung. Die Masse jedes Zylinders sind: Höhe 68 Å, Durchmesser 20.5 Å.

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