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Hydrodynamic properties of α– globulin from *Sesamum indicum* L.

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Abstract. The protein α-globulin from *Sesamum indicum* L. has been characterised for its size and shape using various chemical, physico-chemical and hydrodynamic properties. The protein has an $S^0_{20,w}$ of 12·8, $D_{20,w}$ of 4·9 × 10^{-7} cm^2/sec and a partial specific volume of 0·725 ml/g in the native state. The intrinsic viscosity of the protein was determined to be 3·0 ml/g indicating it to be globular in shape. The molecular weight of the protein as determined by various approaches in analytical ultracentrifugation varies from 2·6–2·74 × 10^5. The molecular weight from sedimentation equilibrium yields a value of 2·74 × 10^5 in the native state and a value of 19000 in the dissociated and denatured state in 6 M guanidine hydrochloride. The evaluation of frictional ratios using Stokes radius and results from electron microscopy confirms the protein to be globular in shape. The protein consists of at least 12–14 subunits. The evaluation of hydrophobic parameters and energetics of interaction of subunits indicate that the protein is stabilized predominantly by hydrophobic interactions.

Keywords. α-Globulin; subunits; sesame seed; hydrophobic.

Introduction

Physicochemical characterization of seed proteins is important in order to understand the nature of the protein, its interaction with other cell components and more importantly the energetics of the stability of the molecule. The major protein of *Sesamum indicum,* α-globulin, constitutes ~ 65–70 % of the total proteins present in the seed (Prakash and Nandi, 1978). The protein has been isolated and characterized (Nath *et al.,* 1957; Sinha and Sen, 1962; Ventura and Lima, 1963; Prakash, 1976). The association-dissociation and denaturation behaviour of α-globulin under various solution conditions of electrolytes, detergents, urea, guanidine hydrochloride (GuHCl), acid and alkali are studied in detail (Prakash and Nandi, 1976a, b, 1977a, b, c, 1978, 1982; Prakash, 1980, 1982; Prakash *et al.,* 1980; Lakshmi and Nandi, 1977, 1978). Recently it was shown that α-globulin is rich in aperiodic and *ß*-structure (Prakash, 1980; Prakash *et al.,* 1980; Prakash and Nandi, 1982).

In the present study in order to determine the probable size and shape of α-globulin, the protein isolated by a nondenaturing procedure has been studied from a hydrodynamic angle and several physical parameters such as molecular weight, diffusion co-efficient, Stokes radius, partial specific volume, intrinsic viscosity,

Abbreviations used: GuHCl, Guanidine hydrochloride; NPS, nonpolar side chains; SDS, sodium dodecyl sulphate; PAGE, Polyacrylamide gel electrophoresis.

sedimentation coefficient and subunit composition are determined. These parameters have been used to understand the various forces which hold the subunits of α-globulin as a thermodynamically stable protein unit.

Materials and methods

Sesame seeds of authentic variety were obtained commercially and α-globulin was isolated from the defatted flour (Prakash and Nandi, 1978).

The chemicals and reagents used were as follows and the source of these materials is shown in the parenthesis: Sepharose 6B-100, 2-mercapto ethanol, GuHCl (Sigma Chemicals); sodium chloride and potassium hydrogen phosphate (BDH Chemicals).

α-Globulin was isolated in a homogeneous form by the previously described method (Prakash and Nandi, 1978). Solutions of α-globulin in 0·1 M phosphate buffer pH 6·5 containing 1 M NaCl (PS buffer) were prepared and used in all the experiments unless otherwise stated.

Determination of protein concentration

Protein concentrations were measured routinely by ultraviolet absorption using a value of 9·54 dl/g-cm as the extinction coefficient of α-globulin in PS buffer (Prakash, 1982).

Velocity sedimentation

Velocity sedimentation experiments were carried out in PS buffer and also in 6 M GuHCl in a spinco Model E analytical ultracentrifuge. A standard 12 mm single sector Kel F Centerpiece was used, and the experiments were carried out at ~ 25°C unless otherwise stated and at 59,780 rpm. Plates were read in a Gaertner microcomporator and $S_{20,w}$ values calculated by standard procedure (Schachman, 1957).

Diffusion experiments

Diffusion experiments were conducted in Spinco Model E analytical ultracentrifuge using a synthetic boundary cell at a rotor speed of 8600 rpm with a protein concentration of 2–12 mg/ml at 25 ± 1°C. Diffusion coefficient was calculated by the height area method described by Kawahara (1969).

Viscosity

Viscosity measurements were made using an Ostwald Viscometer in the presence of 6 M GuHCl at temperature of 30±0·l°C. The intrinsic viscosity determined from extrapolation of reduced viscosities determined at different protein concentrations to zero protein concentration (Yang, 1961; Bradbury, 1970).

Molecular weight determination

Molecular weight of α-globulin was determined from a combination of sedimentation coefficient value and intrinsic viscosity both in buffer and in the presence of 6 M GuHCl using the Scheraga-Mandelkern equation (Scheraga and Mandelkern, 1953). The

molecular weight by Archibald approach to equilibrium and molecular weight by sedimentation and diffusion methods were determined as described by Schachman (1959). The molecular weight by sedimentation equilibrium was determined according to the procedure of Yphantis (1964). Point average molecular weight was calculated both for the native as well as denatured protein in 6 M GuHCl.

Stokes radius

Stokes radius of the protein was determined by gel chromatography in Sepharose 6B-100 column employing 0·04 M sodium phosphate buffer of pH 8·0 containing 5 mM EDTA. The column was equilibrated with the above buffer and calibrated with the standard proteins, urease, bovine serum albumin, ovalbumin, α-chymotrypsin and ribonuclease. The Stokes radius R_s was evaluated by the procedure of Ackers (1967).

Frictional coefficients

The frictional coefficient of α-globulin was calculated by several approaches as reviewed by Siegel and Monty (1966).

Hydrophobicity

The average hydrophobicity and related terms were calculated according to the procedure of Bigelow (1967).

Electron microscopy

The protein samples at low concentration was treated with 0·5% glutaraldehyde, adsorbed to carbon coated formavar grids, stained with 1% uranyl acetate and observed in Philips EM 3001, electron microscope.

Results and discussion

In most of the hydrodynamic studies it is preferable to perform the experiments near the isoelectric point of the protein to reduce the charge effects. Since the solubility of α-globulin was very poor both in buffers of low ionic strength and in buffers of high ionic strength in the region of isoelectric pH (pH 4–5), studies were carried out at pH 6·5 in 0·1 M phosphate buffer containing 1 M NaCl unless otherwise stated *i.e.*, PS buffer. Also the parameter concerned was looked into as a function of protein concentration and the results are tabulated in table 1.

Hydrophobicity and related parameters

The average hydrophobicity and related parameters like frequency of nonpolar side chains (NPS) *i.e.*, nonpolar residue fraction and polar ratio (P) were calculated and are listed in table 1. Both the hydrophobicity and NPS values are high compared to many other oilseed proteins as shown in literature with the concomittant decrease in P value (Bigelow, 1967; Shukla, 1982; Latha and Prakash, 1986). In particular the average hydrophobicity is nearly 100 cals/residue more as compared to carmin, the major

Table 1. Hydrodynamic parameters of α-globulin.

Parameters	Values
1. Hydrophobicity and related values[a]	
(a) Average hydrophobicity	872 cals/residue
(b) NPS	0·26
(c) P	1·36
(d) Charge	0·362 units/residue
2. Sedimentation coefficient ($S^0_{20,w}$)[b]	
(a) Native	12·8 S ± 0·1 S
(b) 6 M GuHCl ($S_{20,w}$)[c]	2·0 S ± 0·2 S
3. Diffusion coefficient ($D_{20,w}$)[d]	$4{\cdot}9 \times 10^{-7}$ cm²/sec
4. Intrinsic viscosity [η][e]	
(a) Native	3·0 ± 0·2 ml/gm
(b) 6 M GuHCl	38·5 ± 1·0 ml/gm
5. Non ideality coefficient (g)[a]	0·01 ml/mgm
6. Partial specific volume[f]	
(a) Native	0·725 ± 0·002 ml/gm
(b) 6 M GuHCl	0·684 ± 0·002 ml/gm
7. Hydrated volume (Ve)[g]	0·711 ml/gm
8. Hydration factor (ϕ)[h]	0·27 gm of water/gm of protein
9. Molecular weight	
(a) Approach to sedimentation equilibrium[i]	2,50,000 ± 20,000
(b) Sedimentation and diffusion measurement[j]	2,36,000 ± 15,000
(c) Sedimentation and intrinsic viscosity[k]	2,52,000 ± 15,000
(d) Sedimentation equilibrium[l]	
(i) Native Mn (number average)	2,65,000 ± 12,000
Mw (weight average)	2,74,000 ± 14,000
(ii) 6 M GuHCl	
Mw (number average)	18,000 ± 1,000
Mw (weight average)	19,000 ± 1,500
10. Size	
(a) Stokes radius[m]	37 ± 3 Å
(b) From sedimentation measurements[n,o]	47 ± 4 Å
(c) From diffusion measurements[n,o]	43 ± 4 Å
(d) Radius of gyration[p]	29 ± 3 Å
11. Frictional ratios[n,o]	
(a) From radius of equivalent sphere	1·00 ± 0·05
(b) From sedimentation and molecular weight	1·10 ± 0·1
(c) From Stokes radius and hydrated volume	1·00 ± 0·15
12. Shape parameters[q,r]	
(a) β-function	$2{\cdot}18 \times 10^6$
(b) Perrin shape factor	1·50 ± 0·1
(c) Simha shape factor	4·17 ± 0·5
13. Axial ratios[q,r]	
(a) Prolate ellipsoid of revolution	3·5
(b) Oblate ellipsoid of revolution	3·0

Table 1. (*Contd.*)

Parameters	Values
14. Subunit number	
(a) SDS-PAGE[i]	**12 (6 × 2) (minimum)**
(b) 6 M GuHCl[l]	**14 (7 × 2)**
15. Subunit interactions	**Predominantly hydrophobic**

[a]BigeSow (1967); [b,j,k]Schachman (1957); [l]Prakash and Nandi (1977a); [d]Kawahara (1969); [e]Bradbury (1970); [f]Prakash (1982); [g,h]Haschemeyer and Haschemeyer (1973); [i]Prakash and Nandi (1977b; 1978); [j]Yphantis (1964); [m]Ackers (1967); Tanford *et al.,* (1974); [o]Siegel and Monty (1966); [p]Pilz (1973); [q]Simha (1940); [r]Scheraga and Mandelkern (1953).

protein of safflower seed protein (Latha and Prakash, 1986). Carmin has an NPS value of 0·2 and charge of 0·609 units/residue. These results indicate that α-globulin is rich in hydrophobic amino acids and possibly many of its properties are governed by this high content of hydrophobic amino acids like, solubility, heat coagulation, subunit-subunit interactions etc. However one has to be cautious in using these empirical hydrophobicity values with the structure-function relationships.

Velocity sedimentation

The sedimentation coefficient determined as a function of protein concentration is shown in figure 1. From the graph $S^0_{20,w}$ value of 12·8 ± 0·1 was obtained for α-globulin. The concentration dependence showed the usual negative slope and was analysed according to the standard procedure (Schachman, 1959). The analysis yielded a value for non-ideality coefficient g = 0·01 ml/mgm (Schachman, 1957).

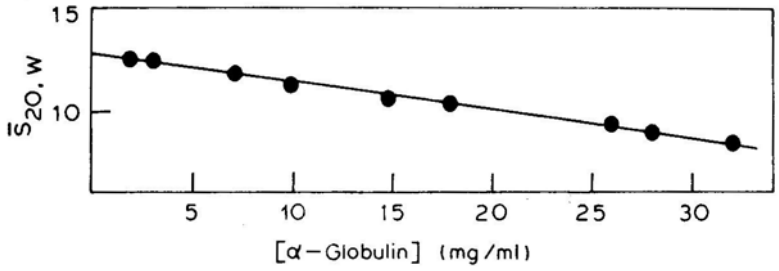

Figure 1. Concentration dependence of the $S_{20,w}$ value of α-globulin in PS buffer.

Viscosity

Figure 2A shows a plot of reduced viscosity as a function of protein concentration over the concentration range of 0·9–5·8% of α-globulin in PS buffer. Interpolation of the results to zero protein concentration gives a value of 3·0 ml/gm for the intrinsic viscosity of α-globulin, in PS buffer suggesting a globular shape for the protein α-

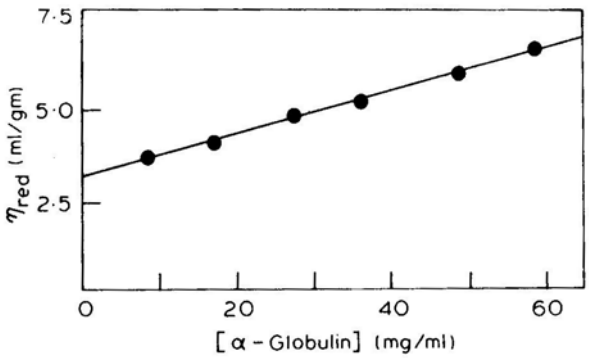

Figure 2. Concentration dependence of η_{red} of α-globulin in PS buffer.

globulin in the native state (Tanford, 1961). Prakash and Nandi (1977)also reported a value of 3·0 ml/gm as the viscosity of α-globulin in 0·02 M phosphate buffer of pH 7·5 containing 1 M NaCl. In figure 3 is shown the plot ofreduced viscosity *versus* protein concentration in 6 M GuH Cl for which an intrinsic viscosity of 38·5 ml/gm is obtained. This value is in the region for random coils as reported by Tanford (1968).

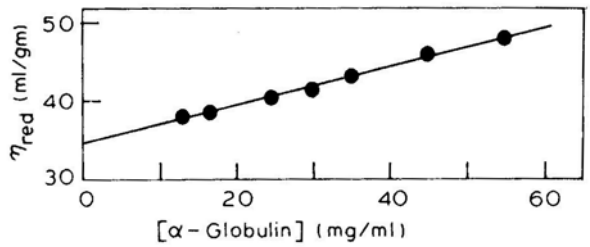

Figure 3. Concentration dependence of n_{red} of α-globulin in 6 M GuHCl containing 0.1 M of 2-mercaptoethanol.

Table 1 lists the molecular weights calculated by various hydrodynamic approaches. The values indicate the protein to be of high molecular weight of around 0·27 million. From the results one can see that a combination of sedimentation and diffusion measurements gives the lowest value of $2{\cdot}36\times10^5$ whereas sedimentation equilibrium in native state shows the highest value of $2{\cdot}74\times10^5$ (figure 4A), others being in between these two values (table 1). Figure 4A shows a plot of *In* (*f*) *versus* r^2 of the sedimentation equilibrium pattern ofα-globulin in native condition. The straight line through all the points till the bottom of the cell and the closeness of the number and weight average molecular weight indicates theprotein to be highly homogeneous with a weight average molecular weight of $2{\cdot}74\times10^5$. Since sedimentation equilibrium has a firm thermodynamic basis the value obtained from it *i. e.*, $2{\cdot}74\times10^5$ is taken as the apparent molecular weight of the protein for the purposes of calculations. The

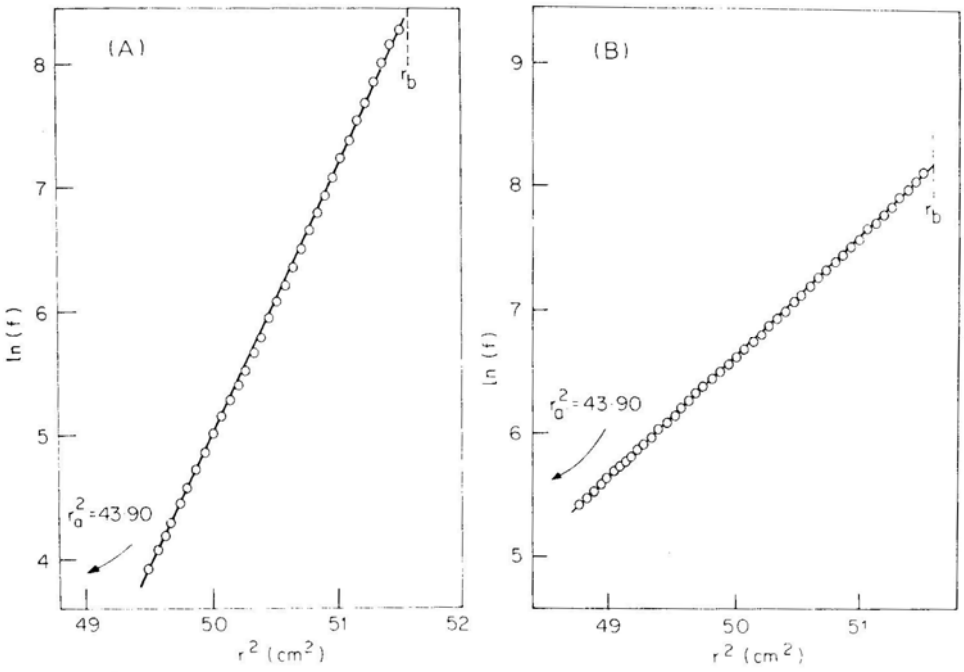

Figure 4. Sedimentation equilibrium patterns of α-globulin in (a) PS buffer and (b) in 6 M GuHCl respectively containing 0.1 M of 2-mercaptoethanol at 20°C. The speed ofthe run and the concentration of the protein used respectively is indicated against each photograph. The results are plotted as *In* of fringe displacement *versus* (radial distance)^2, (a) 12,000 rpm; 480/μg/ml; (B) 32,000 rpm; 300μg/ml.

molecular weight of other oilseed proteins from various sources are also in the same range (Prakash and Narasinga Rao, 1984).

Stokes radius and frictional ratios

Stokes radius of the protein was determined experimentally from gel filtration and gave a value of 37 A. It was also calculated from the experimentally determined sedimentation and diffusion values by using the equations described by Tanford *et al.* (1974). The values obtained *i. e.,* 47A and 43A respectively from sedimentation and diffusion are higher as compared to the experimentally obtained Stokes radius. The approximate radius of gyration was also calculated by using the appropriate equation for a sphere (Pilz, 1973) and gave a value of 29 A. R_{min} was also calculated assuming α-globulin to be a nonhydrated sphere. This gave a value of 43 A which is in excellent agreement with the value calculated from diffusion measurements and also not too far from the experimentally determined Stokes radius value.

Frictional ratios were evaluated from the sedimentation, partial specific volume, molecular weight, intrinsic viscosity and hydrated volume by using the appropriate equation. The values are shown in table 1. All the values suggest a frictional coefficient

close to 1·0 indicating the protein to be compact and globular in shape. These results confirm the earlier results of intrinsic viscosity. From the Scheraga and Mandelkern (1953) equation the value of β-function was calculated and it yields a value of $2{\cdot}18 \times 10^6$ from which one can envisage on axial ratio for prolate ellipsoid of resolution of 3·5 and for oblate ellipsoid of resolution of 3·0 respectively. Other relevant shape parameters are also given in table 1.

Subunit composition

The subunit composition of the protein as determined using sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) in the presence of a disulfide reducing agent indicates at least 12 subunits, in the molecular weight range of 8000-85000 (Prakash and Nandi, 1978). On this basis, one can tentatively conclude that the oligomer is made up of the least 12 nonidentical subunits. Sedimentation equilibrium data in 6 M GuHCl in the presence of 2-mercaptoethanol (figure 4) indicate a weight average molecular weight from the straight line fit of the In (f) *versus* r^2. plot, of 19000 and a number average of 18000. If the native molecular weight is taken as $2{\cdot}74 \times 10^5$, a total number of 14 subunits can be envisaged for α-globulin each of molecular weight 19000. These results are incontradictionto the SDS-PAGE results in terms of the range of molecular weights and also the number of subunits. The anomaly in SDS-PAGE may be due to the presence ofnearly 1·0 % carbohydrate in the protein as reported by Prakash and Nandi (1978). Also Segrest *et al.* (1971) and Neville (1971), have indicated the anomalous mobility and behaviour of proteins containing carbohydrates in the SDS-PAGE systems. Further, since figure 4B indicates a straight line with no curvature till the bottom of the cell, the system is homogeneous in itself and the subunits are all probably of equimolecular weight. Further the homogeneity of the system is well established since the number and weight average molecular weights are close to each other within experimental limitations. This is not unusual in the quaternary structure of many proteins whose subunits are of equal molecular weight (Haschemeyer and Haschemeyer,1973).

Nature of subunit interaction

α-Globulin exists as a single component in 0·5 M Tris/HCl buffer of pH 7·0 with sedimentation coefficient of 13 S (Prakash and Nandi, 1977c). Increase in the pH induces dissociation and at pH 9·0, the 13 S component at 27°C dissociates to nearly 40 % to an 8 S component (Prakash and Nandi, 1977c). The effect of variation of temperature at pH9·0 indicates that increase in temperature favours the associated 13 S component and suggests qualitatively, hydrophobic interactions of the subunits of α-globulin (Prakash and Nandi, 1977c).

To characterise and quantify the thermodynamic parameters and the nature of the association reaction in α-globulin the following procedure was adopted. From the ratio of concentration of 13 S to 8 S the apparent association constant K for the association reaction at three different temperatures were calculated. A plot of log K as a function of reciprocal of absolute temperature is found to be linear as has been represented in figure 5. From the slope of this plot, a value of 13 Kcal/mol is obtained for the enthalpy

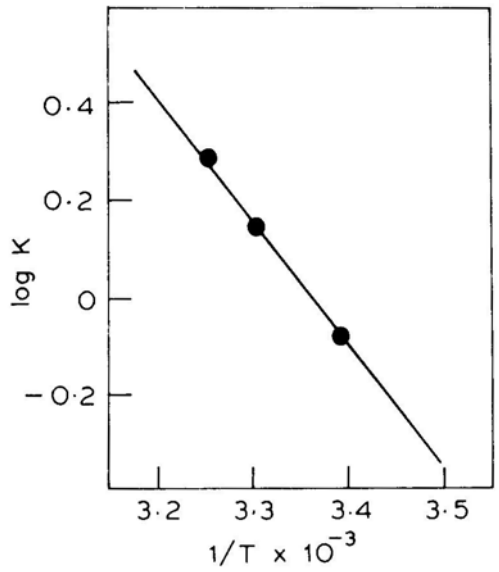

Figure 5. Plot of log *K versus* 1/*T* where *K* is the apparent association constant of 13 S component and *T* the absolute temperature.

of the association reaction. The free energy

$$\Delta F = -RT \ln K, \quad (1)$$

where *R* is the gas constant, *T* the absolute temperature and *K* the apparent association constant gives a value of —0·19Kcal/mol at 27°C. These results yield a value of Δ*S* = 42 e.u. for the entropy of the association reaction of 27°C using the equation

$$\Delta F = \Delta H - T.\ \Delta S, \quad (2)$$

where *F* is the free energy, Ä *H* is the enthalpy and Ä*S* is the entropy of the reaction and *T* is the absolute temperature. This indicates that the association of the subunits of the protein α-globulin is predominantly governed by hydrophobic interactions (Kauzmann, 1959).

Electron microscopy

Figure 6 shows the photomicrograph of α-globulin in the electron microscope. A close examination of the photomicrograph indicates cluster or beads or individual beads as shown by arrows and indicates the protein to be fairly globular in shape. The absence of other structure like rods, or ellipsoids confirms the already available data that the protein is globular in shape.

It is important to keep in mind that the dimensions of protein described in this study are only approximations assuming either spherical or equivalent ellipsoidal models. These should be interpreted with caution in describing the real particles.

The sedimentation coefficient calculated for α-globulin is well within the range for globular proteins of molecular weight of $2{\cdot}5\times10^5$. Even if we assume 1/3 hydration, the

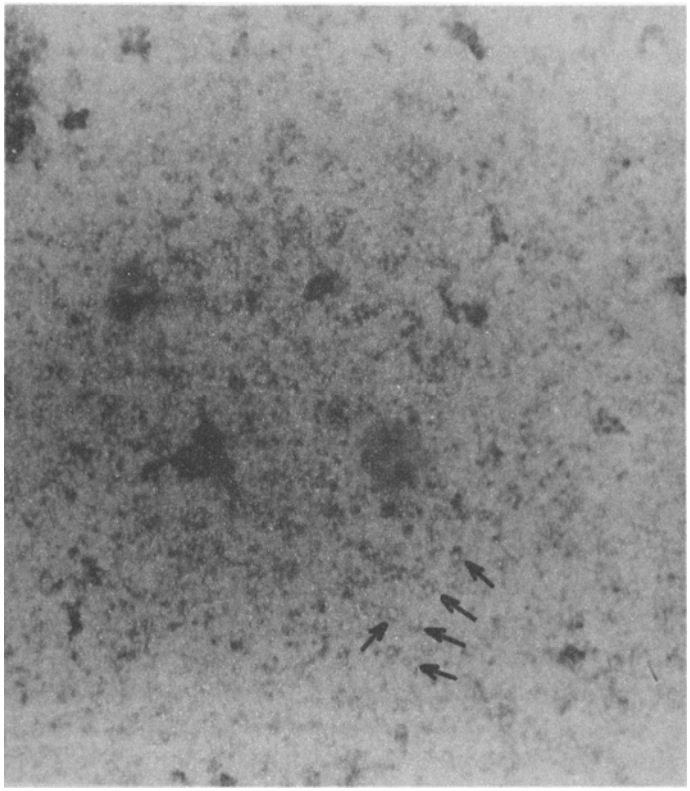

Figure 6. Photomicrograph of α-globulin in the electron microscope at 10000 times magnification factor.

observed $S^{0}_{20,w}$ is not far of from the range of globular shape for α-globulin. The hydration factor appears to be high (table 1) and this may be due to the high compactness of the protein molecule as reflected by [*n*] of 3·0 ml/gm and as also the high content of hydrophobic amino acids and the clathrate formation around these apolar groups (Jencks, 1969).

The exact interpretation of the overall shape of the protein *α*-globulin depends very much on the definition of the limiting axial ratio, intrinsic viscosity and the comparison of the various radii along with their respective molecular weights. In conclusion, α-lobulin is a highly compacted globular protein of molecular weight 274,000 and is made up of at least 12–14 subunits of equal molecular weights of ~ 19000 predominantly held together by entropically driven hydrophobic interactions with a negative free energy of interaction of 0·19 Kcal/mol.

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