Molecular Weights and Volumes from Density Perturbation Ultracentrifugation. Application to Aldolase and Deoxyribonucleic Acid Polymerase in Solutions of Guanidine Hydrochloride*

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ABSTRACT: Sedimentation equilibrium experiments are described which yield both molecular weights and apparent partial specific volumes. The basis of the measurements is the simultaneous examination of protein solutions in natural and heavy water. The analysis is applied to both two-component systems (protein; dilute buffer) and three-component systems (protein, water; guanidine hydrochloride) using rabbit muscle aldolase and *Escherichia coli* DNA polymerase. Studies of the native proteins yielded molecular weights of 154,000 ± 2000 for aldolase and 115,000 ± 5000 for polymerase with partial specific volumes, $\bar{v}$, in the range 0.74 ± 0.01 cc/g in both cases. In solutions of guanidine hydrochloride (or deuterioguanidine deuteriochloride), the effect of preferential interactions is significant, with the apparent partial specific volume, ϕ', of aldolase ranging from 0.70 cc/g in 3 M to 0.74 cc/g in 7 M solutions. For the polymerase, ϕ' varies from 0.72 cc/g in 3 M to 0.76 cc/g in 7 M solutions. The values of ϕ' are derived from an interaction parameter, q_1, defined in terms of the volume of water bound to or excluded from the protein. The values of this parameter are inherently imprecise due to the nature of the analytical procedure. This imprecision results in uncertainties of about 5% in the values of ϕ', and about 15% in the molecular weights of the proteins in the guanidine solutions. However, a greater degree of accuracy can be obtained by averaging several series of experiments. Such analysis yields a value for the molecular weight of aldolase in the guanidine solutions of 37,000, which is consistent with the presence of four polypeptide chains. A value of 110,000 was found for DNA polymerase in the guanidine solutions, providing further evidence that this protein is composed of a single polypeptide chain.

The effective molecular weight, $M(1 - \bar{v}\rho)$, of a macromolecule can be determined conveniently and accurately from sedimentation equilibrium experiments with the analytical ultracentrifuge and interference or absorption optical systems (Yphantis, 1964; Schachman and Edelstein, 1966; Richards *et al.*, 1968). However, in all cases an independent measurement of $\bar{v}$ is necessary, and frequently the determination of this parameter is more demanding, in terms of material and effort, than the sedimentation equilibrium experiment itself. One approach to simplify the $\bar{v}$ analysis has been to design a method based on density perturbation with D_2O (or $[^{18}O]D_2O$) which yields $\bar{v}$ from a comparison of the sedimentation equilibrium distributions of a macromolecule in natural and heavy water (Edelstein and Schachman, 1967; Edelstein, 1967).

When sedimentation equilibrium experiments are conducted in high concentrations of guanidine hydrochloride, or in other three component solutions, $\bar{v}$ is no longer an appropriate quantity to use in expressing the effective molecular weight because of interactions between the protein and the solvent. An apparent partial specific volume, ϕ', may be defined such that the effect of these interactions is cancelled (Casassa and Eisenberg, 1961, 1964), with the effective molecular weight expressed as $M(1 - \phi'\rho)$. Several procedures for measuring apparent partial specific volumes have been developed. A method for determining ϕ' from density increments at constant chemical potential (Casassa and Eisenberg, 1961, 1964) has been widely used (Kielley and Harrington, 1960; Woods *et al.*, 1963; Marler *et al.*, 1964; Reisler and Eisenberg, 1969). Kirby-Hade and Tanford (1967) developed a procedure for determining ϕ' from isopiestic compositions. Noelken and Timasheff (1967) employed light scattering and refractometry to determine ϕ' in guanidine hydrochloride solutions, techniques with Timasheff (1970) has extended to other solvent systems. All of these procedures suffer from the disadvantage that large amounts of material are required since the concentration must be known. This paper describes the extention of the density perturbation method of ultracentrifugation to the determination of ϕ' with microgram quantities of material, based on the assumption that interactions are essentially the same in H_2O and D_2O on a volume basis.

Materials and Methods

Rabbit muscle aldolase was purchased from C. F. Boehringer und Soehne; Gu-HCl[1] was a Mann Ultra Pure product, and D_2O (99.5%) was obtained from Matheson, Coleman & Bell. Gu-DCl was prepared by the following procedure. A 4 M solution of Gu-HCl in D_2O was equilibrated overnight, then evaporated *in vacuo*, and dried over P_2O_5 overnight. The distillate was collected and its density determined.

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[1] Abbreviations used are: Gu-HCl, guanidine hydrochloride in protonated solutions; Gu-DCl, deuterioguanidine deuteriochloride in deuterated solutions; guanidinium chloride, both protonated and deuterated solutions.

From the density, the extent of deuteration could be determined.

$$\% \text{ deuteration} = \frac{\rho_{Dis} - \rho_{H_2O}}{\rho_{D_2O} - \rho_{H_2O}} \times 100$$

where ρ_{Dis}, ρ_{D_2O}, and ρ_{H_2O} are the densities of the distillate, pure D_2O, and pure H_2O, respectively. This procedure resulted in 75.6% deuteration. Solutions 3, 5, and 7 M in this Gu-DCl were prepared using D_2O, and were, respectively, 96, 93, and 89% deuterated. The per cent deuteration was calculated from the weights of Gu-DCl and D_2O used in preparing the solutions.

Sedimentation equilibrium experiments in H_2O and D_2O were performed simultaneously using two cells containing Yphantis centerpieces and sapphire windows in a Spinco Model E ultracentrifuge. Column heights of 2.5 mm were routinely employed.

Rotor speeds were chosen such that the meniscus depletion condition described by Yphantis (1964) applied. Native aldolase was examined at 20,000 rpm, and aldolase in the presence of guanidinium chloride was examined at 40,000 rpm. Solutions of the polymerase were centrifuged at several speeds, ranging from 20,000 to 32,000 rpm. The data were collected using the photoelectric scanner and multiplex attachment; light with a wavelength of 280 nm was used with a photomultiplier slit of 0.1 mm and a monochromator slit of 2 mm.

All of the experiments were conducted at 20° on solutions containing 0.2 mg/ml of protein. Native aldolase solutions also contained 0.1 M phosphate (pH 7.0). Solutions of native polymerase contained 0.1 M KCl and 2.5 mM potassium phosphate (pH 6.8). Solutions of Gu-HCl (or Gu-DCl) contained, in addition to the protein, 0.01 M mercaptoethanol. The densities of the solutions were determined using a 0.4-ml pycnometer and a Mettler microanalytical balance.

Theory

The concentration gradient of a macromolecule in water or a dilute buffer (assuming an ideal solution and electroneutrality) at equilibrium in a centrifugal field is described by

$$\frac{d \ln C_2}{dr^2} = \frac{M_2\omega^2(1 - \bar{v}_2\rho)}{2RT} \quad (1)$$

where C_2, M_2, and $\bar{v}_2$ are, respectively, the concentration, molecular weight, and partial specific volume of the macromolecule; r is the distance from the axis of rotation, ω is the angular velocity, R is the gas constant, T is the absolute temperature, and ρ is the density of the solution. Ideality is assumed in this treatment since all solutions to be considered are very dilute.

When a third component (component 1 is water; component 2, the macromolecule; component 3, a low molecular weight salt) is present in appreciable concentration, interactions between protein and solvent must be considered. The net effect of these interactions on the apparent molecular weight is termed a preferential interaction, and is usually defined in terms of a set of interaction parameters; $\xi_1 = (\partial w_1/\partial w_2)\mu$ and $\xi_3 = (\partial w_3/\partial w_2)\mu$, where ξ_1 (or ξ_3) is the number of grams of component 1 (or 3) which must be added per gram of component 2 to maintain components 1 and 3 at constant chemical potential.

The effect of these interactions on molecular weight determinations may be eliminated by defining an apparent partial specific volume, ϕ' (Casassa and Eisenberg 1961, 1964), such that

$$(1 - \phi'\rho) = (\partial\rho\partial C_2)\mu = \quad (2a)$$

$$(1 - \bar{v}_2\rho) + \xi_1(1 - \bar{v}_1\rho) \quad (2b)$$

In terms of this parameter, the concentration gradient of a macromolecule in a three-component solution is described by the equation

$$\frac{d \ln C_2}{dr^2} = \frac{M_2\omega^2(1 - \phi'\rho)}{2RT} \quad (3)$$

To determine ϕ', a method similar to that described by Edelstein and Schachman (1967) for determining $\bar{v}_2$ and M_2 in two-component solutions may be used. To determine $\bar{v}_2$ and M_2, parallel sedimentation equilibrium experiments were conducted in H_2O and D_2O solutions. Deuterium exchange between protein and solvent was taken into account, and two equations of the form of eq 1 were generated. These equations were then solved simultaneously to yield $\bar{v}_2$ and M_2.

If the density perturbation method is used to determine the quantities M_2 and ϕ' in eq 3, errors are introduced since ϕ' is a function of density (eq 2b). Equation 3 must be solved in terms of M_2 and a parameter which is density independent. A volumetric interaction parameter will fit this criterion if it is assumed that H_2O and D_2O solutions interact with a protein to the same extent on a molar basis (see Discussion), since the molar volumes of H_2O and D_2O are nearly the same (18.0991 and 18.1589, respectively, at 30° (Steckel and Szapiro, 1963)). The volumetric interaction parameter, q_1, may be defined as

$$q_1 = (\partial V_1/\partial w_2)\mu \quad (4)$$

$$= \xi_1/\rho_1$$

where V_1 is the volume, and ρ_1 is the density of component 1. When expressed in terms of q_1

$$\phi' = \bar{v}_2 + q_1(1 - \rho_1/\rho) \quad (5)$$

and

$$\frac{d \ln C_2}{dr^2} = \frac{M_2\omega^2[(1 - \bar{v}_2\rho) + q_1(\rho_1 - \rho)]}{2RT} \quad (6)$$

For experiments conducted in D_2O, eq 6 must be corrected to account for replacement of exchangeable hydrogens of the protein by deuterium exchange. If a quantity, k, is defined as the ratio of the molecular weight of the deuterated to that of the nondeuterated macromolecule, the molecular weight of a macromolecule in a deuterated solution will attain the value kM_2, and the partial specific volume will become $\bar{v}_2/k$. Hence in D_2O, the concentration gradient is described by the equation

$$\frac{d \ln C_2}{dr^2} = \frac{kM_2\omega^2[(1 - \bar{v}_2\rho/k) + q_1(\rho_1 - \rho)]}{2RT} \quad (7)$$

TABLE 1: Quantities Derived from Density Perturbation Ultracentrifugation of Aldolase in Guanidinium Chloride–0.01 M Mercaptoethanol at 20°.[a]

Concn of Gu-HCl or Gu-DCl	3 M	5 M	7 M
ρ_H	1.075	1.120	1.164
ρ_D	1.173	1.213	1.246
$M_2(1 - \phi'_2\rho) \times 10^{-3}$			
H_2O	9.6 ± 0.1	7.4 ± 0.2	5.8 ± 0.2
D_2O	7.3 ± 0.3	5.4 ± 0.2	4.0 ± 0.1
$M_{2,app} \times 10^{-3}$			
H_2O	47 ± 1	44 ± 1	43 ± 1
D_2O	51 ± 2	48 ± 2	45 ± 1
q_1	−0.6 ± 0.6	−0.4 ± 0.4	−0.1 ± 0.1
ϕ'	0.70 ± 0.04	0.70 ± 0.04	0.73 ± 0.01
$M_{2,true} \times 10^{-3}$	39 ± 6	34 ± 6	39 ± 4

[a] Each set of data is the average of four experiments; the errors represent average deviations. See the text for details of calculations.

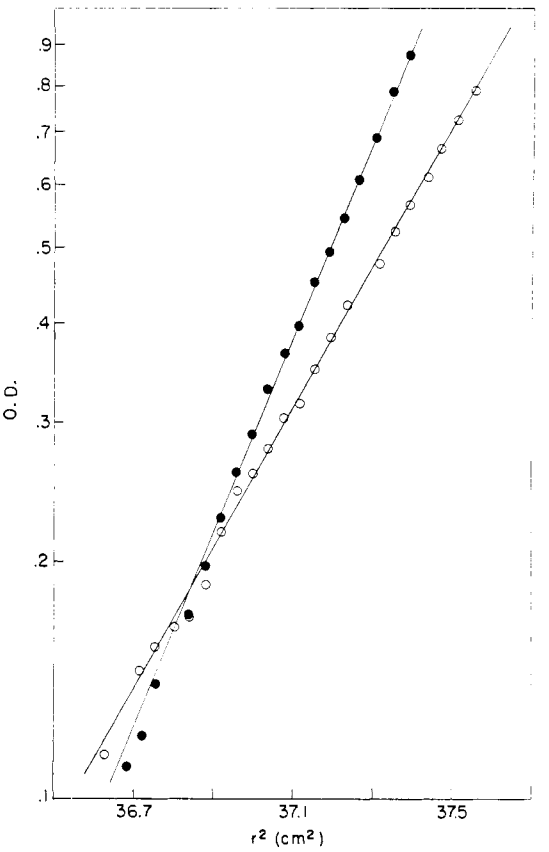

FIGURE 1: Equilibrium sedimentation of native aldolase in H_2O and D_2O. Solutions contained 0.2 mg/ml of aldolase in 0.1 M phosphate in H_2O at pH 7 (●) or in D_2O at pD 7 (○). The rotor was maintained at 20°; the speed was 20,000 rpm.

Since the exchangeable hydrogens reside principally in the polypeptide backbone (one hydrogen per amino acid) and to a much lesser extent in the amino acid side chains, the value of k can be considered relatively constant for all proteins. The value $k = 1.0155$ (Martin *et al.*, 1959; Hvidt and Nielsen, 1966) has been determined for a number of proteins in 100% D_2O. For concentrations of D_2O below 100%, the value of k is reduced proportionately.

Equations 6 and 7 may be solved for q_1.

$$q_1 = \frac{\bar{v}_2(\rho_D - F\rho_H) + F - k}{k(\rho_{D_2O} - \rho_D) - F(\rho_{H_2O} - \rho_H)} \quad (8)$$

where $F = (\mathrm{d} \ln C_2/\mathrm{d}r^2)_{D_2O}/(\mathrm{d} \ln C_2/\mathrm{d}r^2)_{H_2O}$; ρ_D and ρ_H are, respectively, the densities of the D_2O and H_2O solutions; and ρ_{H_2O} and ρ_{D_2O} are the densities of pure H_2O and pure D_2O (or H_2O–D_2O mixture), respectively. Substituting this result into eq 5 for the parameters in H_2O

$$\phi' = \frac{v_2(kJ - \rho_D) + k - F}{kJ + F\rho_H} \quad (9)$$

where

$$J = \frac{\rho_H(\rho_{D_2O} - \rho_D)}{(\rho_H - \rho_{H_2O})}$$

Results

The partial specific volume and molecular weight of native aldolase were determined by the density perturbation method of Edelstein and Schachman (1967). Figure 1 illustrates plots of the data in log C *vs.* r^2 form from an experiment conducted in H_2O and one conducted in D_2O. From such plots, values of d ln $C_2/\mathrm{d}r^2$ were measured, and effective molecular weights calculated. An effective molecular weight in H_2O, $M_2(1 - \bar{v}_2\rho)$, of 38,200 ± 600, and an effective molecular weight in D_2O, $M_2(k - \bar{v}_2\rho)$, of 28,600 ± 600 were determined as the average of four experiments. Using these effective molecular weights, and the values 1.013 and 1.118 as the densities of the H_2O and D_2O solutions, respectively, the partial specific volume and molecular weight were calculated: $\bar{v}_2 = 0.742 \pm 0.005$; $M_2 = 154{,}000 \pm 2000$.

The results of experiments performed on solutions of aldolase in concentrated Gu-HCl are presented in Table I. The effective molecular weights (rows 3 and 4) were calculated from the slope of log C *vs.* r^2 plots of the data, which showed no detectable curvature. In Figure 2, representative plots are presented in which the effect of the increased density of the deuterated solutions can be seen. The apparent molecular weights (rows 5 and 6) were calculated using eq 1 and the values $\bar{v}_2 = 0.742$ and $k = 1.014$. As can be seen by comparing the apparent molecular weight in H_2O to the apparent molecular weight in D_2O, the determination of the interaction parameter, q_1, is based on a difference of only about 10%. Large errors in q_1 should therefore be expected. The values of q_1 presented in row 7 were calculated using eq 8, and the following values: $\bar{v}_2 = 0.742$, $\rho_{H_2O} = 0.9983$, $\rho_{D_2O} = 1.100$, $k = 1.014$. The apparent partial specific volume, ϕ', was calculated using eq 5. The values for the true molecular weight given in the last row were calculated using eq 3. As can be seen, the true molecular weight is about 20% less than the apparent molecular weight.

The partial specific volume and molecular weight of native *E. coli* DNA polymerase were determined by the density

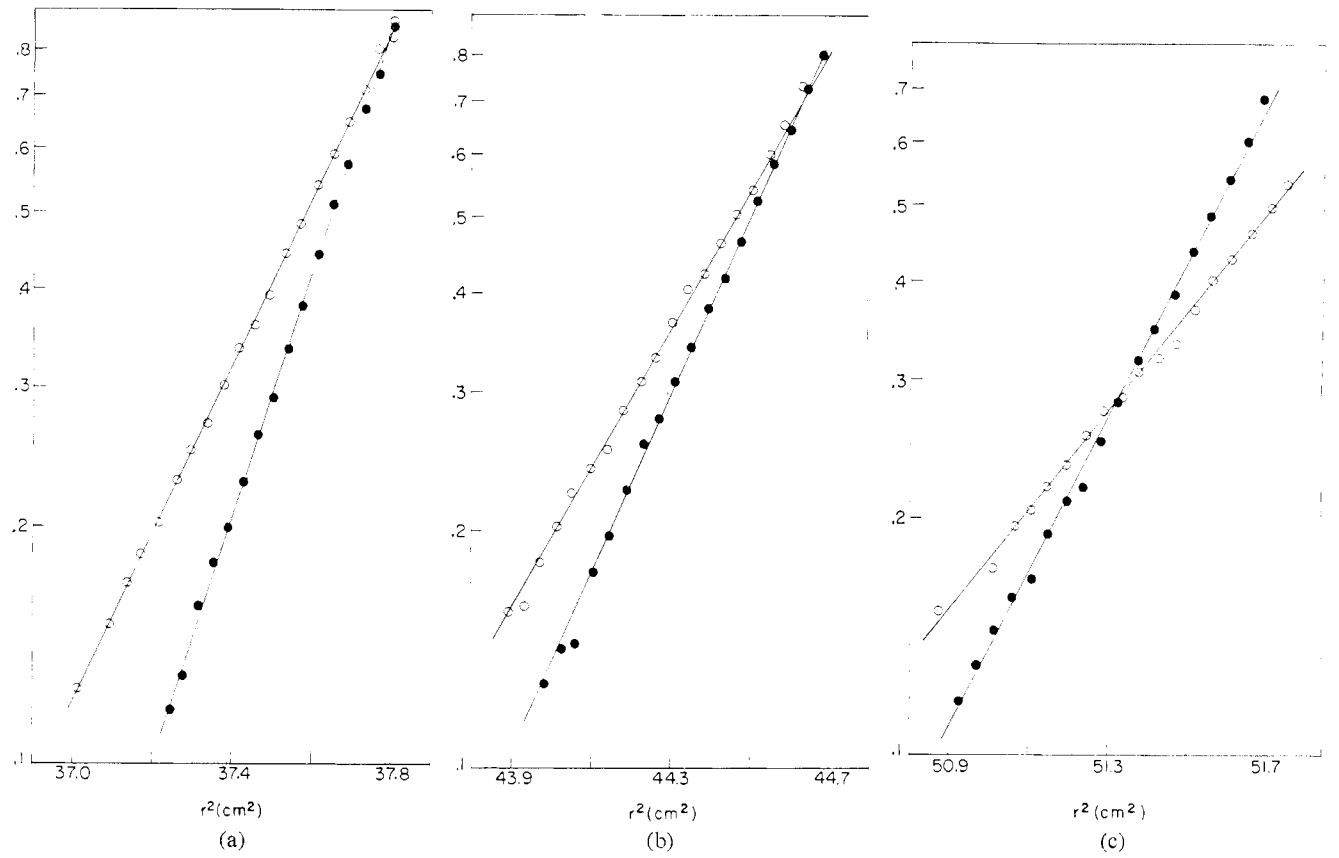

FIGURE 2: Equilibrium sedimentation of aldolase in solutions of Gu-HCl and Gu-DCl. Solutions contained 0.2 mg/ml of aldolase in protonated (●) or deuterated (○) solvents. (a) 3 M guanidinium chloride, (b) 5 M guanidinium chloride, and (c) 7 M guanidinium chloride. The rotor was maintained at 20°; the speed was 40,000 rpm.

perturbation method of Edelstein and Schachman (1967). The values $\bar{v}_2 = 0.74 \pm 0.01$ and $M_2 = 115{,}000 \pm 5000$ were determined as the average of three experiments.

A summary of density perturbation experiments performed on the polymerase in 3, 5, and 7 M solutions of Gu-HCl is presented in Table II. The results were calculated as described for aldolase. The values $\bar{v}_2 = 0.745$ (Jovin *et al.*, 1969), $\rho_{D_2O} = 1.090$, $\rho_{H_2O} = 0.9983$, and $k = 1.014$ were used in the calculations. The results indicate a true molecular weight in the area of 110,000. A problem with heterogeneity was encountered in some of the experiments as evidenced by a large upward curvature in the log C *vs.* r^2 plots near the bottom of the cell. The data from these experiments were not used in compiling Table II.

Discussion

The determination of preferential interactions by density perturbation ultracentrifugation is based on a difference of about 10% between the apparent molecular weight determined in H_2O and that determined in D_2O (Tables I and II). The experimental error of about 3% in the determination of the quantity d ln C_2/dr^2 is, therefore, magnified to an error of about 100% in the determination of q_1. The large errors in q_1 are, however, of significance only to the extent that they influence the molecular weight determination. As shown by eq 5, the influence of preferential interactions on the partial specific volume is a function of both q_1 and the ratio ρ_1/ρ. As this ratio approaches one, the effect of q_1 diminishes. In these experiments, the ratio is sufficiently close to one (even in 7 M Gu-HCl, $\rho_1/\rho = 0.861$), to reduce the large error in q_1 to a much smaller error in ϕ' and M_2. These errors are, however, still significant; a 1% error in the quantity d ln C_2/dr^2 will result in a 5% error in the molecular weight. To minimize this error, the results of several experiments should be averaged.

In calculating the apparent partial specific volume, $\bar{v}_2$ is multiplied by the quantity $kJ - \rho_D$ (eq 9), which is about 0.2 in these experiments. Therefore, only an approximate value of $\bar{v}_2$ need be known. The value of $\bar{v}_2$ determined for the native protein, or a value calculated from the amino acid composition is adequate, particularly in light of studies which have shown that $\bar{v}_2$ does not change appreciably in going from the native to the denatured state (Linderstrøm-Lang, 1949; Charlwood, 1957; Reithel and Sakura, 1963; Katz and Ferris, 1966).

Attention must be given to the homogeneity of the macromolecule. If the H_2O and D_2O solutions are examined at the same speed, the macromolecule will sediment to a much greater extent in the H_2O solution than in the D_2O solution. At any one point in the cell, therefore, the effect of heterogeneity will be different in D_2O than in H_2O. For this reason, when dealing with a heterogeneous system, care must be taken to examine the entire length of the cell. Alternatively, the H_2O and D_2O solutions can be run at different speeds such that $(\text{d ln } C_2/dr^2)_{D_2O} = (\text{d ln } C_2/dr^2)_{H_2O}$.

The interaction parameters determined in these experiments are compared in Figure 3 to those determined by other workers using various methods and proteins. In addition to their determination of ξ_1 for aldolase, as shown in Figure 3,

TABLE II: Quantities Derived from Density Perturbation Ultracentrifugation of *E. coli* DNA Polymerase in Guanidinium Chloride–0.01 M Mercaptoethanol at 20°.[a]

Concn of Gu-HCl or Gu-DCl	3 M	5 M	7 M
ρ_H	1.070	1.116	1.159
ρ_D	1.164	1.203	1.239
$M_2(1 - \phi'\rho) \times 10^{-3}$			
H_2O	23.1 ± 0.6	18.2 ± 0.2	13.9 ± 0.3
D_2O	17.6 ± 0.6	12.8 ± 0.2	8.9 ± 0.3
$M_{2,app} \times 10^{-3}$			
H_2O	114 ± 3	108 ± 1	100 ± 2
D_2O	120 ± 4	108 ± 2	97 ± 3
q_1	−0.4 ± 0.5	0.0 ± 0.2	+0.1 ± 0.1
ϕ'	0.72 ± 0.03	0.74 ± 0.02	0.76 ± 0.01
$M_{2,true} \times 10^{-3}$	100 ± 15	105 ± 15	115 ± 15

[a] Each set of data is the average of four experiments; the errors represent average deviations. See the text for details of calculations.

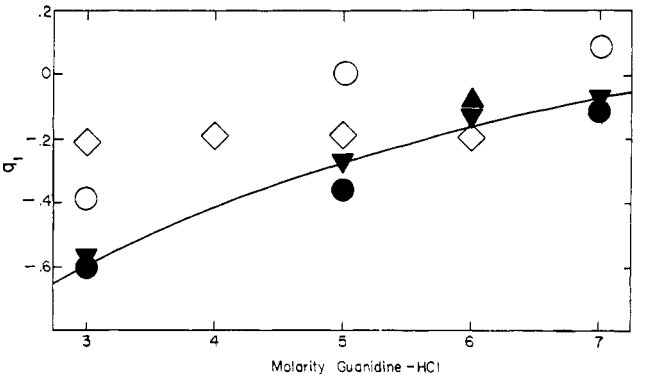

FIGURE 3: Interaction parameters of various proteins in guanidine hydrochloride. Values of q_1 were calculated from reported values of ϕ' using eq 5 and the reported value of $\bar{v}_2$, or from reported values of ξ_1 or ξ_3 using the relation $q_1 = \xi_1/\rho_1 = -1000\ \xi_3/m_3M_3\rho_1$ (Kirby-Hade and Tanford, 1967; Noelken and Timasheff, 1967). (◇) Bovine serium albumin, determined from light scattering and refractometry by Noelken and Timasheff (1967); (○) DNA polymerase, this study; (▼) aldolase, determined from density increments by Reisler and Eisenberg (1969); (●) aldolase, this study; (▲) aldolase, determined from isopiestic compositions by Kirby-Hade and Tanford (1967).

Kirby-Hade and Tanford (1967) measured the preferential interactions in 6 M Gu-HCl of several other proteins, obtaining values for $\xi_1(\xi_1 = q_1/\rho_1)$ between −0.07 and −0.11 with the exception of ribonuclease, for which $\xi_1 = +0.004$. It is of interest to note that for both ribonuclease and DNA polymerase, two proteins which interact with nucleic acids, the values for ξ_1 are somewhat higher than for other proteins. This difference must, however, be interpreted with caution in view of the large errors involved in the measurements. Considering the large degree of error involved in determining preferential interactions, the agreement between our results and those of other workers is quite good. This agreement supports the assumption upon which this method is based; that preferential interactions between protein and deuterated solvent are not significantly different from the preferential interactions in the protonated solvent.

Schachman and Edelstein (1966) determined q_1 to be +0.14 for aldolase, and Jovin *et al.* (1969) obtained a value of +0.25 for DNA polymerase from experiments in which plots of the effective molecular weight, $M_2(1 - \phi'\rho)$, in solutions of varying concentration of Gu-HCl *vs.* the density of these solutions were extrapolated to the density at which no redistribution would occur. The extrapolation is a long one, and makes the assumption that ϕ' and q_1 are independent of composition. Reisler and Eisenberg (1969) have shown, and our data verify that this assumption is not valid.

The effective molecular weights, $M_2(1 - \phi'\rho)$, of aldolase determined in H_2O solutions (Table I), are in good agreement with those determined by Schachman and Edelstein (1966), who obtained values of 10,070, 7820, and 5590 for 3, 5, and 7 M solutions of Gu-HCl. From these values, they calculated a true molecular weight of 50,000, consistent with a three subunit model of aldolase, using the apparent partial specific volume $\bar{v}_e = 0.775$, determined by their method of extrapolation. Using the apparent partial specific volumes in Table I, a true molecular weight of 37,000 is calculated. This value is consistent with a four-subunit model of aldolase, the molecular weight of the native protein being 155,000. Since the hybridization experiments of Penhoet *et al.* (1967) favoring a four subunit model, the evidence has left little doubt that the four subunit model is the correct one. The results obtained with H_2O solutions of the polymerase differ with those obtained by Jovin *et al.* (1969), who report apparent molecular weights of 100,000, 90,000, and 85,000 for 3, 4.5, and 6 M solutions of Gu-HCl with 0.3 M mercaptoethanol. The value $\bar{v}_2 = 0.745$ used in their calculations is the same as that used in this paper. Using these values and the interaction parameters listed in Table II, values for the true molecular weight of about 90,000 are obtained. In view of the evidence that the polymerase is a monomer with a molecular weight of 110,000 (Jovin *et al.*, 1969), this value is somewhat low. The true molecular weight calculated from the data in Table II is 110,000, which is in good agreement with a monomer model.

This method provides the means by which a complete ultracentrifugal analysis can be performed with only microgram quantities of material. Although this study has been restricted to proteins in guanidine hydrochloride solutions, the method is readily applicable to experiments conducted in nondissociating solvents such as sodium chloride (Edelstein and Gibson, 1969).

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Structural Studies on the Duck 5.7S and 7.8S Immunoglobulins*

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ABSTRACT: Structural studies on the duck 5.7S and 7.8S immunoglobulins revealed the former to have a molecular weight of 118,000, an Fab fragment of 48,000, and component heavy and light chains of 35,000 and 23,000, respectively. The carboxy-terminal residue on the heavy chain was phenylalanine and the molecule had a relatively low total carbohydrate content of 0.6%, which was localized to the Fab fragment. The 7.8S immunoglobulin was shown to have a molecular weight of 178,000 with a heavy chain of 62,000–66,000. The total carbohydrate content was 5.0%. Both proteins are structurally distinct from previously described mammalian immunoglobulins. The phylogenetic implications of these findings with respect to immunoglobulin evolution are discussed.

The study of nonmammalian γ-globulins has provided a useful tool for the analysis of immunoglobulin evolution. Avian antibodies which differ in certain respects from mammalian have been studied extensively in two species, the duck and the chicken (Leslie and Clem, 1969; Dreesman and Benedict, 1965; Tenenhouse and Deutsch, 1966). In the duck two classes of low molecular weight immunoglobulins exist, the biologic properties of which have been investigated and reported previously (Grey, 1967a,b). In brief, following immunization with bovine serum albumin, both 7.8S and 5.7S antibovine serum albumin antibodies were found, although late in the immune response the 5.7S antibody predominated. The 7.8S antibodies fixed duck complement and fixed to duck skin whereas the 5.7S antibody gave negative results in both these systems. Experiments were also performed to rule out generation of the 5.7 S molecule through degradation of the 7.8 S. Injection into ducks of isotopically labeled 7.8S protein demonstrated that the 5.7S immunoglobulin was not a breakdown product of the larger protein.

In this paper we describe some of the major structural characteristics of these two low molecular weight immunoglobulins.

Materials and Methods

Protein Isolation. Adult Pekin and Muscovy male ducks were immunized by weekly intramuscular injections of 1 mg of bovine serum albumin in complete Freund's adjuvant. Sera from these ducks were fractionated in two steps (Grey, 1967a,b): starch block electrophoresis followed by application of the γ-globulins to an upward-flow Sephadex G-200 or Bio-Gel P300 column from which they were eluted with phosphate-buffered saline.

Antigenic Analysis of γ-Globulins. Antisera were prepared in rabbits against whole duck serum and purified γ-globulins by repeated footpad injections of 1 mg of the antigen in complete Freund's adjuvant. Ouchterlony gel diffusion (Ouchterlony, 1953) and immunoelectrophoresis (Scheidegger,

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