MOLECULAR WEIGHT STUDIES ON CONCANAVALIN A

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SUMMARY. Molecular weight studies have been performed on solutions of Concanavalin A (Con A) at pH values of 5.2, 7.0, and 7.5. The results suggest that Con A exists as a mixture of a species with molecular weight of 30,000 g/mole and higher aggregates. At pH 5.2 dimers predominate resulting in whole cell weight average molecular weights of approximately 55,000 g/mole, while at neutral pH, trimers are indicated, yielding molecular weights of the order of 90,000 g/mole.

Electrophoresis on sodium dodecyl sulfate polyacrylamide gels showed the presence of one major band with molecular weight 30,000 - 35,000 g/mole and two minor ones with M values of 13,000 - 15,000 and 21,000 - 23,000 g/mole, respectively. Equilibrium centrifugation of maleyl Con A, and Con A in 5 M guanidine HCl yielded a subunit molecular weight close to 17,000 g/mole. The two species plot of Roark and Yphantis suggested a species of molecular weight 13,000 - 15,000 g/mole in these media.

If the preparative molecular weight of Con A is assumed to be around 70,000 g/mole then our results suggest that the molecule can exist as "half-mers" and under appropriate conditions, these can further dissociate into the basic subunit of ∿17,000 g/mole. A logical inference of these findings is that Con A is apparently composed of four subunits.

The molecular weight of the crystalline jack bean protein Concanavalin A (Con A) was initially reported to be 96,000 g/mole (1). Agrawal and Goldstein (2) reported a molecular weight of 68,000 g/mole for a protein similar in many of its properties to Con A described by Sumner and Howell (3), but which was isolated by sorption on Sephadex rather than by crystallization. In both cases, molecular weight was calculated from sedimentation and diffusion rate measurements. Gel filtration studies on Bio-Gel P-100 estimated the molecular weights of Con A as 40,000 g/mole, 71,000 g/mole and >100,000 g/mole at pH values of 2.2, 7.0, and 10.2 respectively (4). A recent study employing the technique of low speed sedimentation equilibrium revealed that the apparent weight average molecular weight was invariant over the pH range 3.5 - 5.8, the actual

value being 55,000 g/mole. Above pH 5.8, both in Tris and phosphate buffers the weight average molecular weight rose steeply and erratically, figures from 80,000 g/mole - 100,000 g/mole being obtained at pH values around 7 (5).

It is apparent from these observations that a high degree of uncertainty still surrounds the size and molecular weight of Con A, and it was with a view to clarification of this problem that the present study was initiated.

We wish to present a summary of sedimentation (velocity and equilibrium) and viscosity data on Con A at pH 5.2 in 0.2 M NaCl, 0.02 M sodium acetate and sedimentation data at pH 7.0 and 7.5 in .2 M NaCl, 0.01 M KPO_4 and 0.01 M Tris HCl, respectively. The protein was dissociated by treatment with maleic anhydride, or high concentrations of guanidine hydrochloride, and physico-chemical measurements on the resulting subunits are also described. Data from sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis studies are also presented.

EXPERIMENTAL. Con A was obtained from Miles-Yeda Ltd. in 30% saturated ammonium sulphate suspension. This protein was 3x crystallized and showed a single band on polyacrylamide gel electrophoresis at pH 4.5. Solutions for experiments were prepared by dialyzing small aliquots of the above suspension against the preferred buffer system.

Formation of protein subunits was accomplished by dialysis against 5 or 6 M guanidine HCl solutions or with maleic anhydride at pH 9 (6), followed by extensive dialysis to remove excess reagent.

A Beckman model E analytical ultracentrifuge was used for sedimentation velocity and equilibrium studies. The latter studies were performed using the Rayleigh interference optical system with methodology described by Richards and Schachman for low speed experiments (7) and that of Yphantis for meniscus depletion ones (8). Molecular weight measurements in high concentrations of guanidine HCl were

performed at 20°C by the meniscus depletion method, using a 7 mm column and layering the solvent on to the protein solution in a synthetic boundary interference cell. In this way times to equilibrium were considerably reduced. SDS polyacrylamide gel electrophoresis was performed by the method of Dunker and Rueckert (9). Viscosity measurements were carried out at 20°C as outlined previously (10).

RESULTS AND DISCUSSION

Studies at pH 5.2: Sedimentation velocity measurements in 0.2 M NaCl, 0.02 M sodium acetate revealed the presence of a single peak with an $S^{\circ}_{20,w}$ value of 3.80 ± 0.14 with the equation of the regression line being $S = S^{\circ}(1-k,c)$ where $k = 1.33 \times 10^{-2}$ ℓ/g. Viscosity measurements yielded an intrinsic viscosity, [η], of 0.041 ± 0.0005 dℓ/g. The linear plot of ηsp/c versus c was essentially horizontal.

Fig. 1 shows a plot of weight average molecular weight as a function of protein concentration. This plot was drawn from low speed, and meniscus depletion experiments at several different initial loading concentrations. It

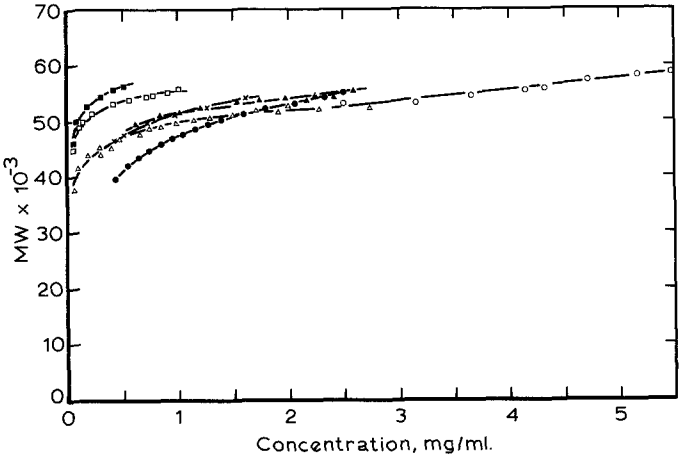

Fig. 1. Weight average molecular weight as a function of protein concentration for Con A at 20°C in 0.2 M NaCl, 0.02 M sodium acetate, pH 5.2. ■ represents an initial concentration of 0.25 mg/ml, □, 0.35 mg/ml, x, 0.87 mg/ml, ▲, 1.30 mg/ml, Δ, 1.1 mg/ml, ●, 1.06 mg/ml, and o, 3.25 mg/ml. Low speed runs were carried out at 10,000 or 12,000 r.p.m. Meniscus depletion experiments were performed at 24,000 or 26,000 r.p.m.

is apparent from this curve that if cell weight average molecular weights are calculated over the concentration range of ∿2 - 6 mg/ml then a figure of 55,000 g/mole results. Below 1 mg/ml there is a pronounced decrease in molecular weight as the concentration decreases. At present, due mainly to the variability in results at very low concentration, we have been unable to extrapolate this plot to infinite dilution with any reliability. As one possible approach to this problem we have applied the Roark and Yphantis "two species" plot (11) to data from meniscus depletion experiments. Plots of $M_{w\,(r)}$ versus $1/M_{n(r)}$ were linear and from the points of intersection with the hyperbola $\frac{M_w}{M_n} = 1$, the monomer and n-mer molecular weights could be established. We assumed ideal behaviour in the low protein concentration range employed. This approach yielded a monomer species of molecular weight around 30,000 g/mole and suggested that the other species was dimer as illustrated in Fig. 2.

In an attempt to answer the question of possible dissociation at very low concentrations, meniscus depletion experiments were carried out with

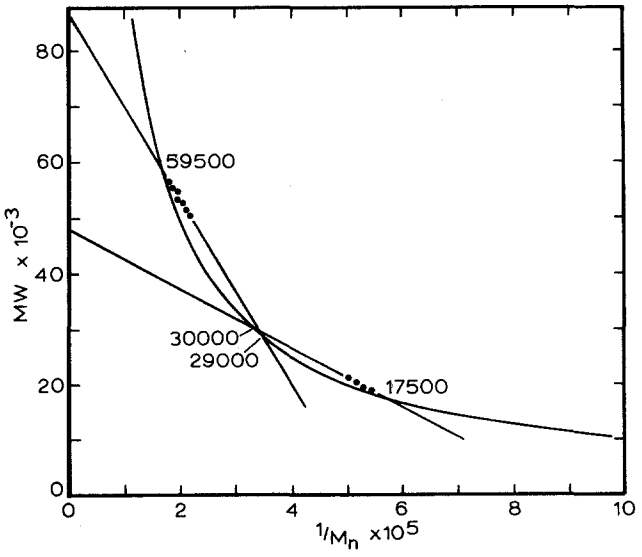

Fig. 2. Two-species plots for Con A at pH 5.2 and maleyl Con A. The numbers at the intersections of the lines with the hyperbola, give the molecular weights of the various species present.

initial loading concentrations in the range 0.25 mg/ml - 0.50 mg/ml. It was noted that there was not good overlap in the plots of molecular weight versus concentration, as the initial concentration varied. Part of this discrepancy may lie in our application of the method at these very low concentrations and also from the computer curve fitting process, which calculates point weight average molecular weights, as this is possibly subject to "end" effects. Occasionally at these low concentrations data has been obtained suggesting linear ℓnc versus r^2 plots and constant values for molecular weight in the range 50,000 - 53,000 g/mole.

Harris et al. (12) in a study of yeast aldolase noted a non overlap in their molecular weight versus concentration curves and attributed it to a non equilibrating mixture of monomer and dimer. Assuming then that the fall off in molecular weight noted for Con A at low concentration is real, then the problem arises to find the value at infinite dilution. Unfortunately as yet we have not obtained precise enough data at these low concentrations to make any definitive statement. As stated above the "two species" plot suggested a mixture of monomer (M = 30,000) and dimer. More extensive work to answer these questions is presently under way.

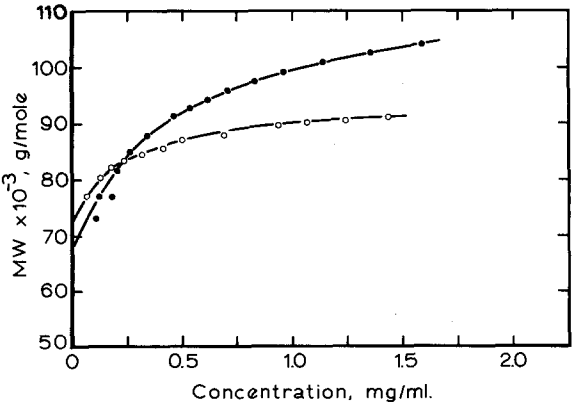

Fig. 3. Weight average molecular weight as a function of protein concentration for Con A at 20°C, meniscus depletion experiments at 20,000 r.p.m. ●, represents an initial concentration of 0.25 mg/ml, in 0.01 M Tris-HCl, pH 7.5, o, 0.5 mg/ml, in 0.2 M NaCl, 0.01 M $K[PO_4]$, pH 7.

Studies at pH 7.0 and 7.5: Sedimentation velocity measurements either in 0.2 M NaCl, 0.01 M KPO_4, pH 7.0 or 0.01 M Tris HCl, pH 7.5 showed a single peak with $S^{\circ}_{20,w} = 5.80 \pm .10 \times 10^{-13}$ sec and slope term, $k = 1.0 \times 10^{-2}$ ℓ/g.

Fig. 3 shows weight average molecular weight plotted against concentration in the two media. Molecular weights increased very rapidly above protein concentrations of 2 mg/ml in the low ionic strength solvent and therefore most of the discussion will be confined to concentrations below ∿2 mg/ml.

From this data, cell weight average molecular weight calculations result in figures around 90,000 g/mole. Extrapolation of these curves to infinite dilution yielded an intrinsic molecular weight of 70,000 g/mole ± 4,000 g/mole. A "two species" plot indicated a monomer-trimer situation with a monomer molecular weight of ∿35,000 g/mole.

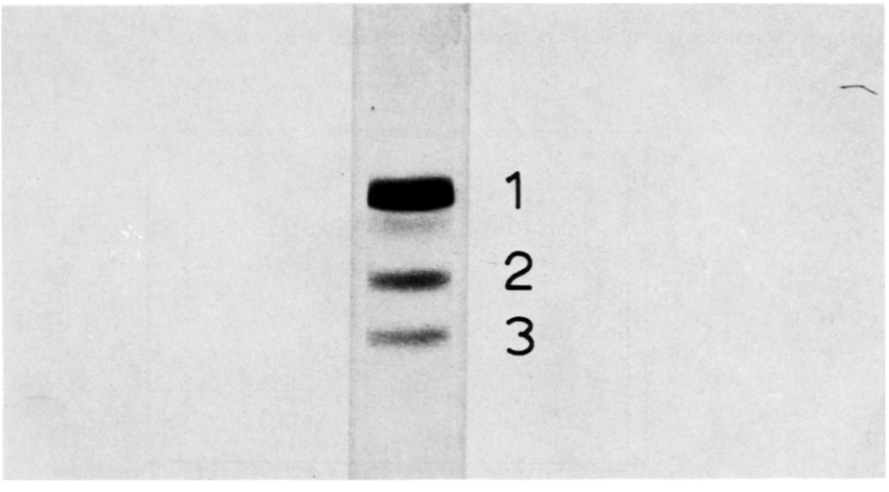

Fig. 4. S.D.S. polyacrylamide gel photograph. 10 μg of Con A applied. Bands 1, 2, and 3 present in relative amounts of 75%, 16%, and 9%, and correspond to molecular weights of 33,000 g/mole, 21,000 g/mole and 15,000 g/mole respectively.

Studies on Sub-Units of Con A: In 1967 Shapiro et al. (13) pointed out that the molecular size of polypeptides could be estimated from relative electrophoretic mobilities of their SDS complexes on polyacrylamide gels. This technique employing 5% gels was used to study Con A. As Fig. 4 shows, Con A exhibits three bands present in relative amounts of 75%, 16%, and 9%. Molecular weights were estimated to be 33,000 g/mole, 21,000 g/mole and 15,000 g/mole respectively. Occasionally a trace (less than 5%) of a band showed up with molecular weight 28,500 g/mole.

Molecular weights in 5 M guanidine HCl, 0.2 M NaCl, 0.02 M sodium acetate solution were measured by a modification of the meniscus depletion technique outlined in the experimental section. Fig. 5 shows apparent weight average molecular weights plotted versus concentration. Extrapolation of these data to infinite dilution yielded a subunit molecular weight close to 17,000 g/mole. A "two species" plot indicated a mixture of species, one with molecular weight 13,000 - 15,000 g/mole and the other close to 24,000 g/mole.

Fig. 6 shows molecular weight versus concentration plots for maleylated Con A, at several different initial protein concentrations in a medium of 0.2 M NaCl, 0.05 M sodium borate, pH 9. Extrapolation of these

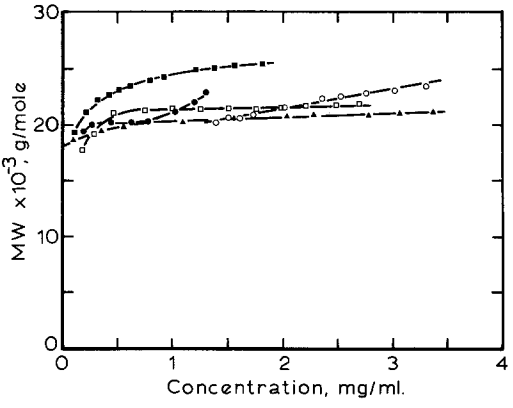

Fig. 5. Weight average molecular weight as a function of protein concentration for Con A at 20°C in 5 M guanidine-HCl, 0.2 M NaCl, 0.02 M sodium acetate. Meniscus depletion experiments at 40,000 r.p.m. ■, represents an initial concentration of 0.5 mg/ml, ●, 1.0 mg/ml, □, 1.2 mg/ml, o, 1.5 mg/ml and ▲, 2 mg/ml.

plots to infinite dilution yielded a subunit molecular weight close to 17,000 g/mole. Solutions of maleylated Con A tended to form aggregates upon dialysis, or storage, and part of the non-overlap in these plots, which were derived using four separate preparations, may reflect the presence of differing amounts of these aggregates. A "two species" plot(Fig. 2) gave evidence for material with molecular weights 17,500 g/mole and 30,000 g/mole. These molecular weight estimates include a contribution from bound maleyl groups. This number has not been determined but from the lysine content of Con A (44 moles/100,000 g protein), it would probably mean a reduction in molecular weight of $\sim$1,000 g/mole.

Viscosity measurements in the guanidine HCl solvent yielded a value of 0.21 ± 0.01 dℓ/g for [η]. Putting this figure for [η] into the empirical equation of Tanford (15), which relates viscosity and chain length of a random coil, we calculate the number of residues "n" to be 167. Using a mean residue weight of 108, this yields a subunit weight of 18,000 g/mole, in good agreement with the direct measurements noted above.

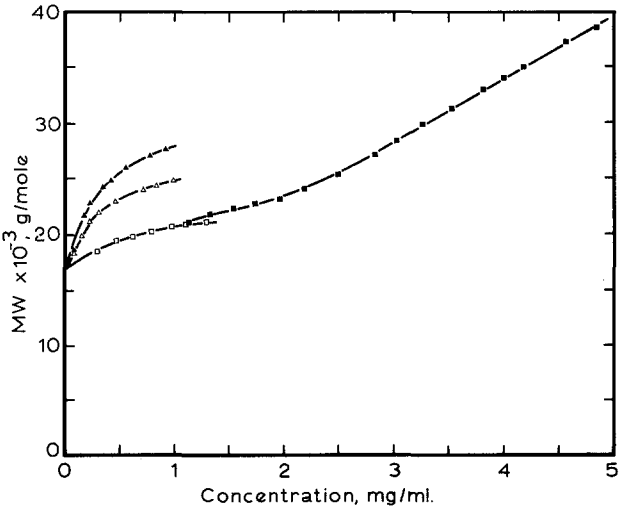

Fig. 6. Weight average molecular weight as a function of protein concentration for maleyl Con A at 20°C in 0.2 M NaCl, 0.05 M sodium borate, pH 9. Meniscus depletion experiments at 40,000 r.p.m. ▲, 0.7 mg/ml, △, 1.14 mg/ml, and □, 1.9 mg/ml. Low speed experiment at 18,000 r.p.m. ■, 2.2 mg/ml.

Olson and Liener (4) concluded from peptide maps of tryptic and peptic digests that Con A is composed of identical subunits. Assuming a preparative weight for Con A, at pH 7 (where it is most active in precipitating dextran) of 71,000 they suggested four subunits of molecular weight close to 16,500 g/mole. Kalb et al. (14) showed that the crystallographic subunit of Con A had a molecular weight of 26,000 g/mole in relatively close agreement with the figure of 30,000 g/mole estimated from equivalent binding weight studies. We would suggest that these latter two forms are possibly dimers of the basic subunit species found in solutions of high concentration of guanidine HCl.

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