Purification and Properties of Guinea Pig Serum Asparaginase*

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ABSTRACT: Guinea pig serum L-asparaginase has been purified *ca.* 900-fold by a combination of sodium sulfate fractionation, gel filtration with G-200 Sephadex, DEAE-cellulose chromatography, and chromatography on calcium hydroxylapatite. The enzyme appears to be homogeneous as judged by several criteria, including sedimentation equilibrium ultracentrifugation and the appearance of a single arc upon immunoelectrophoresis. The molecular weight was found to be *ca.* 138,000 by equilibrium sedimentation, and 133,000 based upon elution volume from G-200 Sephadex. The amino acid composition was determined, and certain properties of GPS asparaginase and *Escherichia coli* B asparaginase, both of which have previously been shown to have antilymphoma activity *vs.* the 6C3HED Gardner lymphosarcoma in C3H mice, have also been compared.

A sevenfold purification of guinea pig serum[1] asparaginase was reported some time ago (Meister, 1955). Interest in this enzyme has increased recently, however, both because of its use in assaying for L-asparagine (Tower *et al.*, 1963) and because the enzyme is the agent in GPS responsible for the antilymphoma activity of the serum, as first suggested by Broome (1961) and later supported by work from our laboratory (Mashburn and Wriston, 1963, 1964; Yellin and Wriston, 1966). Several partial purifications have been reported (Mashburn and Wriston, 1963; Tower *et al.*, 1963; Mardashev and Shao-Khua, 1962; Broome, 1963; Suld and Herbut, 1965). We wish to report here the isolation of a 900-fold purified GPS L-asparaginase which is homogeneous by several criteria, including sedimentation equilibrium ultracentrifugation and immunoelectrophoresis, and to describe certain properties of this enzyme.

Experimental Section

Guinea Pig Serum. Fresh-frozen GPS was purchased from Grand Island Biological Co., Inc. The blood was obtained from fasted inbred guinea pigs (English cavies) of both sexes. It consistently yielded values of 190 or more units of asparaginase/ml, and did not vary appreciably in pH (*ca.* 8) from lot to lot. Serum from another commercial source proved unsatisfactory.

Protein Determination and Asparaginase Assay. Protein concentration was determined according to Waddell (1956) after diluting protein solutions with 0.85% sodium chloride (w/v) or buffer to an optical density at 215 mμ of 1.0 or less (1-cm cell). Measurements were made on the Zeiss PMQ-III spectrophotometer. A standard curve was prepared with crystalline bovine serum albumin (Sigma Chemical Co.). Protein concentration in the effluent from chromatographic columns was usually monitored continuously, either with an Isco ultraviolet monitor at 254 mμ, or with the automatic recording device of Avizonis and Wriston (1962) at 278 mμ.

Asparaginase activity was determined by direct nesslerization. For routine assays, enzyme solution (10–100 μl) was added to 0.1 M sodium borate buffer, pH 8.5, to give a volume of 1.5 ml. The reaction was started by the addition of 0.5 ml of 0.04 M L-asparagine in the same buffer and allowed to proceed for 30 min at 37°. The incubation was stopped by adding 0.5 ml of 15% trichloroacetic acid (w/w) and the incubation mixture was centrifuged, if necessary, for 15 min in a clinical centrifuge. The solution was transferred quantitatively to 10-ml graduated test tubes and diluted to 9 ml with water. It was then combined with 1 ml of Nessler's reagent (Fisher Scientific Co.) in an 18 × 150 Pyrex test tube, and allowed to stand for 15 min at room temperature. Ammonia was estimated by determining the optical density at 500 mμ (for 1–6 μmoles of NH_3), or occasionally at 420 mμ (0.2–2 μmoles of NH_3), with a Spectronic 20 colorimeter. Enzyme and substrate blanks, as well as ammonia standards (2 and 5 μmoles), were included in all assays. Albumin or gelatin was added as a stabilizer in the assays of highly purified asparaginase. A unit of activity is defined as that amount of enzyme which will catalyze the formation of 1 μmole of ammonia/hr under the conditions of

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[1] Abbreviations used in this work: GPS, guinea pig serum; BSA, bovine serum albumin.

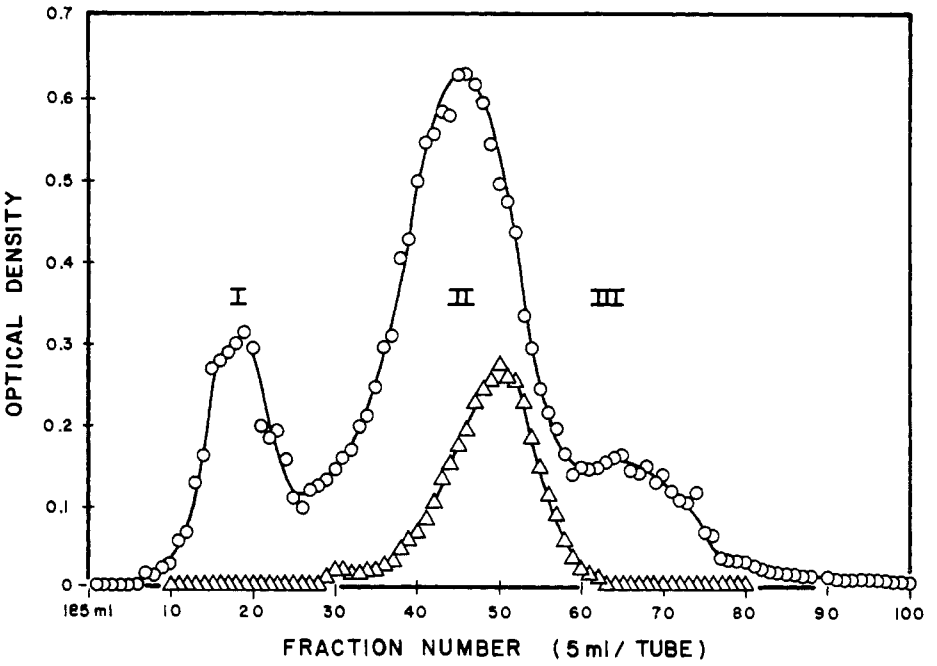

FIGURE 1: Gel Filtration of guinea pig serum protein precipitated with sodium sulfate. G-200 Sephadex was equilibrated with 0.05 M sodium phosphate buffer, pH 8, which was also 1 M in NaCl, and eluted with this same buffer at 30 ml/hr. A forerun of 185 ml was discarded before starting to collect fractions. Protein concentration (open circles) is in terms of optical density at 280 mμ; asparaginase activity (open triangles) is in terms of optical density at 500 mμ (see text for assay).

the assay.[2] Specific activity is expressed as units per milligram of protein.

Chromatography. Sephadex G-200 columns were prepared according to directions supplied by Pharmacia, Inc. The G-200 Sephadex was passed through a U. S. standard 200-mesh sieve before use. DEAE-cellulose (Brown Co., Berlin, N. H., Selectacel, through 325 mesh, 0.9 mequiv/g) was treated according to Peterson and Sober (1962) and columns were packed at 15 psi. The calcium hydroxylapatite was prepared according to the general procedure of Tiselius *et al.* (1956) as described by Levin (1962) and packed at 1.5–2 psi.

Molecular Weight by Gel Filtration. The procedures followed were essentially those of Andrews (1964) who published while this work was in progress. The Sephadex G-200 was hydrated for 72 hr, with decantation to remove fines, before packing. The buffer was 0.05 M Tris–HCl, 1 M NaCl, pH 8, and the column was 2.2 cm in diameter and *ca.* 1 m in height. The effluent was monitored at various wavelengths, usually 278 or 215 mμ, depending on sample concentration, with the use of the automatic recording device of Avizonis and Wriston (1962). Test proteins were dissolved in elution buffer containing 2% sucrose and 4-ml samples were layered beneath the eluent. Flow rate stabilized after <10 ml of effluent had been collected, and was checked periodically. Chart speed was also determined periodically and elution volume (V_e) was determined by measuring the distance to the protein peaks on the chart, converting to milliliters, and adding to the volume collected in volumetric flasks before the chart was started. V_e was also determined directly in volume of effluent collected to the peak tube. Fractions of 3 ml were collected, and in a further check, every fifth fraction was collected in a graduated test tube. It was thus possible to reproduce elution volumes to ±1 ml. The column was calibrated using crystalline bovine serum and its dimer, ovalbumin, and chymotrypsinogen. Test proteins were run alone and in combination, including standards.

Amino Acid Analysis. Protein was hydrolyzed according to Moore and Stein (1963) in 6 N HCl for 22 and 72 hr at 110 ± 1°. Aliquots containing *ca.* 100 μg of protein were analyzed with the automatic amino acid analyzer of Hamilton (1962, 1963) which consists of a single, high-resolution column capable of determining 0.01 μmole of an amino acid to within ±5%. Special precautions were taken to eliminate random contamination (Hamilton, 1965). Values for serine, threonine, and half-cystine were extrapolated to zero time, assuming first-order kinetics. Methionine sulfoxide was added to methionine, and alloisoleucine to isoleucine. Tryptophan was estimated spectrophoto-

[2] The definition used earlier (Mashburn and Wriston, 1963, 1964; that amount of enzyme which will catalyze the formation of 1 μmole of ammonia in 30 min) has been changed to conform to the practice of Meister (1955) and other authors.

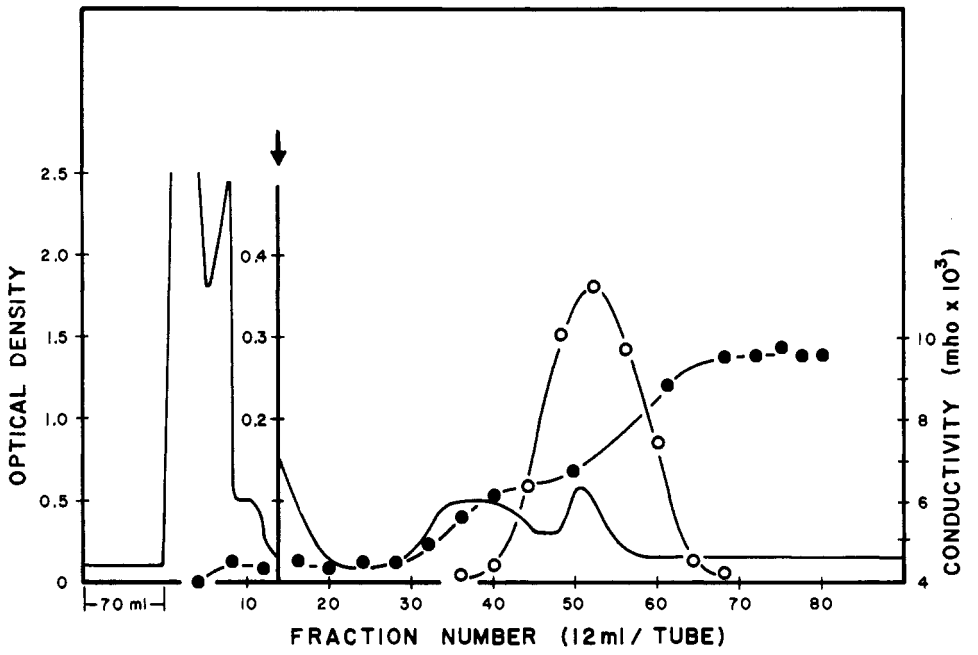

FIGURE 2: DEAE-cellulose chromatography of GPS asparaginase fraction from gel filtration. A 1.8 × 50 cm column was equilibrated with 0.01 M sodium phosphate buffer, pH 8, containing 0.02% NaCl. After *ca.* two column volumes of starting buffer, a concave gradient was started (see text) at the point indicated by an arrow. Protein concentration (solid line) was monitored with an Isco μv monitor at 254 mμ (with a scale change at the point indicated by an arrow). Asparaginase activity is represented by open circles and conductivity by solid circles.

metrically according to Beaven and Holiday (1952). Amide nitrogen was not determined.

Electrophoresis. Immunoelectrophoresis on microscope slides was conducted in the cold room using 0.1 M Tris–HCl buffer (pH 8 at 25°). The Durrum cell, commonly used for paper electrophoresis, was modified for use with agar or polyacrylamide gel slides by making a plexiglass cover 2 cm high to minimize air space. The procedure of Campbell *et al.* (1963) using 0.85% ion agar or 1% agarose was followed.

Polyacrylamide gel electrophoresis was carried out according to Raymond (1962), for 4 hr at 250 v, using EDTA–boric acid–Tris buffer, pH 8.6 (Boyer *et al.*, 1963). The gel was cooled to 0 ± 1° with a circulating water bath, and the voltage turned on for 0.5 hr before sample application.

Sedimentation. Equilibrium ultracentrifugation according to Yphantis (Yphantis, 1964; Filmer and Koshland, 1963) was carried out in the Spinco Model E ultracentrifuge using Rayleigh optics. Rayleigh interference patterns were photographed after centrifuging for 24 hr at 20,410 rpm and 25° in a 12-mm double-sector cell. A Gaertner toolmaker's microscope was used to measure fringe displacements. Sedimentation velocity was determined from the schlieren patterns of the enzyme photographed at 8-min intervals after reaching final speed of 59,780 rpm at 25°. The enzyme was dialyzed for 48 hr *vs.* multiple changes of 0.1 M potassium phosphate buffer, pH 7, for sedimentation experiments.

Concentration of Protein Solutions. Ultrafiltration in the cold room according to Everall and Wright (1958) or Peterson and Sober (1962) was used to concentrate dilute enzyme solution with little or no loss in activity.

Results

Purification of GPS Asparaginase. Fresh frozen GPS (100 ml) was brought to room temperature (20–25°) and stirred gently (magnetic stirrer) for 1 hr after making the serum 2 × 10^{-3} M in DFP, 70 ml of 30% sodium sulfate solution (30 g of anhydrous sodium sulfate in 70 ml of 0.1 M K_2HPO_4) was then added dropwise with continuous stirring, and stirred for 30 min after the addition was completed. The suspension was centrifuged (Servall RC-2, 34,800*g*, 20°, 1 hr) and the pellets dissolved in one-tenth of the original volume of distilled water at room temperature. The enzyme solution was made 5% in sucrose and layered beneath the eluent on a Sephadex G-200 column (3.8-cm diameter, 500–900 ml column volume). The Sephadex was equilibrated with 0.05 M sodium phosphate buffer, pH 8, which was also 1 M in NaCl, and this same buffer was used for elution at a flow rate of 30 ml/hr. The results of a typical gel filtration experiment are shown in Figure 1.

As may be seen, asparaginase was recovered in peak II. When this peak was subjected to paper electrophoresis (Spinco analytrol system) it was found to be composed largely of γ-globulins (*ca.* 87%) with small amounts of β-globulin (10%), α-globulin (3%), and only traces of albumin (most of the GPS albumin re-

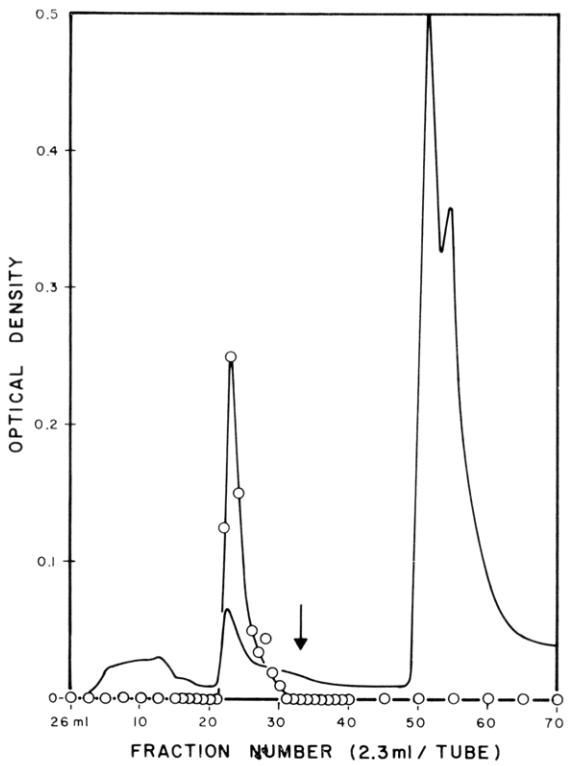

FIGURE 3: Calcium hydroxylapatite chromatography of GPS asparaginase fraction from DEAE-cellulose column. A 2 × 16 cm column was equilibrated with 0.03 M potassium phosphate buffer, pH 6.8. The buffer was changed to 0.08 M potassium phosphate buffer, pH 6.8, at the point indicated by an arrow. A forerun of 26 ml was discarded before starting to collect fractions. Protein concentration (Isco monitor, 254 mμ) is shown by solid lines, and asparaginase activity by open circles.

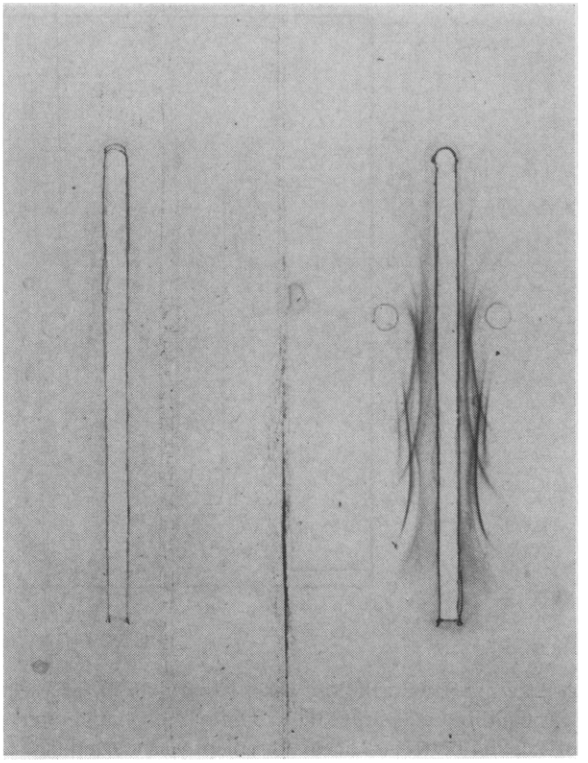

FIGURE 4: Immunoelectrophoresis (see Methods for details). Undiluted GPS was used in the experiment on the right, and purified GPS L-asparaginase from calcium hydroxylapatite chromatography (*ca.* 3 mg of protein/ml) in the experiment shown on the left of the figure.

mains in solution at the sodium sulfate stage). Polyacrylamide gel electrophoresis was also used to follow the course of fractionation.

Flodin and Killander (1962) have shown that when human serum is chromatographed on Sephadex G-200, the first peak contains the macroglobulins and most of the lipoproteins. The apparent molecular weight of peak I protein from GPS was found by gel filtration to be *ca.* 330,000, as may be seen in Figure 7. Peak I was opalescent and stained strongly with Sudan black, while concentrates of peaks II and III did not bind Sudan black, suggesting that here, as with human serum, gel filtration effectively separates macroglobulins and lipoproteins from other serum proteins.

The fractions containing asparaginase were pooled and concentrated by ultrafiltration. The concentrated enzyme solution was dialyzed *vs.* 1 l. of distilled water for 4 hr with continued ultrafiltration, and distilled water was then added to the contents of the dialysis tubing until the conductivity fell below 2×10^{-3} mho. This suspension was centrifuged (Servall RC-2, 34,800*g*, 0°, 30 min) and the turbid supernatant applied to a 1.8 × 50 cm DEAE-cellulose column which had been equilibrated with a 1:4 dilution of stock buffer (0.05 M sodium phosphate, pH 8, containing 1% (w/v) NaCl). Approximately two column volumes of starting buffer were passed through, followed by elution with a concave gradient from starting buffer (250 ml) to a 1:1 dilution of stock buffer (500 ml). The results of a typical separation are shown in Figure 2. Under the conditions used, most of the contaminating protein emerges in the unadsorbed front from the DEAE-cellulose column. Ouchterlony double diffusion experiments with this unadsorbed protein against antiwhole GPS and anti-GPS γ-globulin, as well as immunoelectrophoresis, show it to be pure γ-globulin, presumably 7S. This observation is in accord with that of others (Fahey and Horbett, 1959) on human serum. Fractions containing enzyme were pooled and concentrated by ultrafiltration.

The concentrated enzyme solution from the DEAE column was applied to a calcium hydroxylapatite column (2 × 16 cm, 50–60 ml column volume) which had been equilibrated with 0.03 M potassium phosphate buffer, pH 6.8, and chromatographed by stepwise elution. Hjerten (1959) has shown in the case of human

TABLE I: Purification of GPS Asparaginase.[a]

Step	Volume (ml)	Total Protein (mg)	Total Units	Sp Act. (units/mg)
Guinea pig serum	100	6,400	19,200	2.96
Na_2SO_4	10	1,200	17,300	14.4
G-200 Sephadex	10	624	13,300	21.2
DEAE-cellulose	10	38	4,960	130
Calcium hydroxylapatite	5	0.71	2,000	2,800

[a] Experimental details are given in the text. Recoveries in ultrafiltration and dialysis were essentially quantitative, and these steps are omitted in the table. Over-all recovery was *ca.* 10%.

serum that very little protein is eluted from calcium hydroxylapatite by 0.05 M phosphate buffer at pH 6.8. GPS asparaginase is only slightly retarded on columns equilibrated with 0.03 M phosphate buffer, pH 6.8, however, and consequently is extensively purified by elution with this buffer, as may be seen in Figure 3.

Asparaginase-containing fractions from calcium hydroxylapatite chromatography which are 600–900-fold purified show a single arc on immunoelectrophoresis, as may be seen in Figure 4. The highest specific activity we have obtained is 2800 units/mg representing a 900-fold purification with an over-all recovery of *ca.* 10%. Table I presents a summary of the purification scheme. Variations in specific activity of the material obtained from the calcium hydroxylapatite column are probably due to surface denaturation, as discussed below. Suld and Herbert (1965) found, *e.g.*, that GPS asparaginase which they believed was still not homogeneous had to be assayed immediately after elution from DEAE-cellulose columns because serious losses of activity occurred in a few hours.

Stability. Pure asparaginase was stable for at least 6 months at −20° and to repeated freezing and thawing. It loses activity only slowly at 25° or in the cold room if left undisturbed. The enzyme is also stable to prolonged dialysis *vs.* multiple changes of 0.1 M potassium phosphate buffer, pH 7.0, and to ultrafiltration. The pure enzyme, however, was found to be extremely labile under conditions which promote surface denaturation, as in dilute solutions of low ionic strength, when glass surfaces are present, and especially on incubation with shaking. The data in Table II illustrate this property of the enzyme, and suggest that the purified enzyme should be stored in plastic containers, that shaking and excessive handling be avoided, and that the use of soap or detergent for cleaning glassware to be used with the enzyme be avoided as much as possible.

Molecular Weight. The molecular weight was determined by the method of equilibrium sedimentation according to Yphantis (1964), and the logarithm of fringe displacement plotted against distance from the center of rotation, as shown in Figure 5. The molecular weight was calculated from the slope of the best straight line fitting the experimental points according to the equation

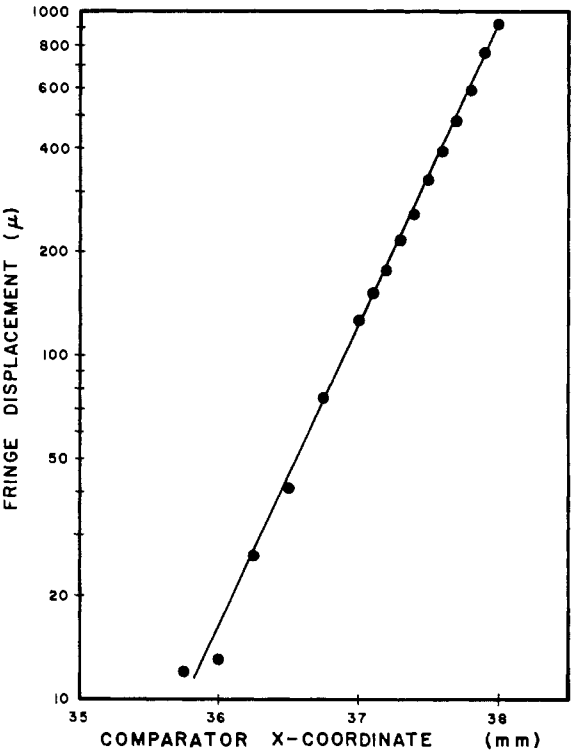

FIGURE 5: Equilibrium sedimentation of GPS asparaginase.

$$M = \frac{RT}{\omega^2 r(1 - \bar{v}\rho)} \frac{\mathrm{d} \ln c}{\mathrm{d}r}$$

The value of $\bar{v}$ was calculated from the amino acid composition of the enzyme (Schachman, 1957) and found to be 0.737. The molecular weight determined by this method (apparent weight-average molecular weight) was 138,265. The linearity of the plot is a strong indication of the homogeneity of the preparation, and additional evidence for homogeneity is provided by the fact that the number-average molecular weight (Yphantis, 1964; eq 5) was found to be 138,000 at several points between one and three fringe displace-

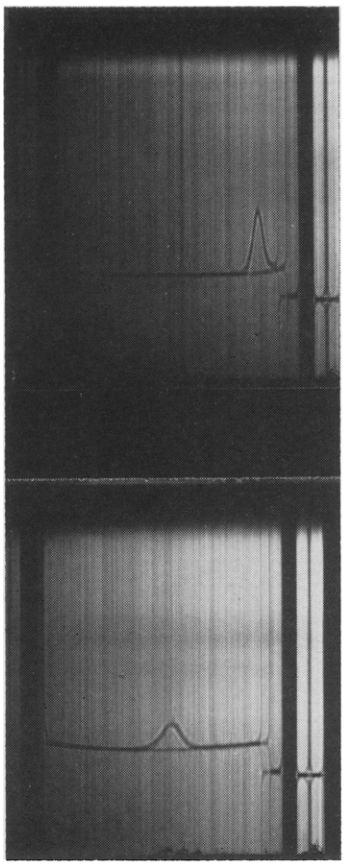

FIGURE 6: Velocity sedimentation of GPS asparaginase. The upper figure was taken 16 min, and the bottom one 54 min, after reaching final speed of 59,780 rpm.

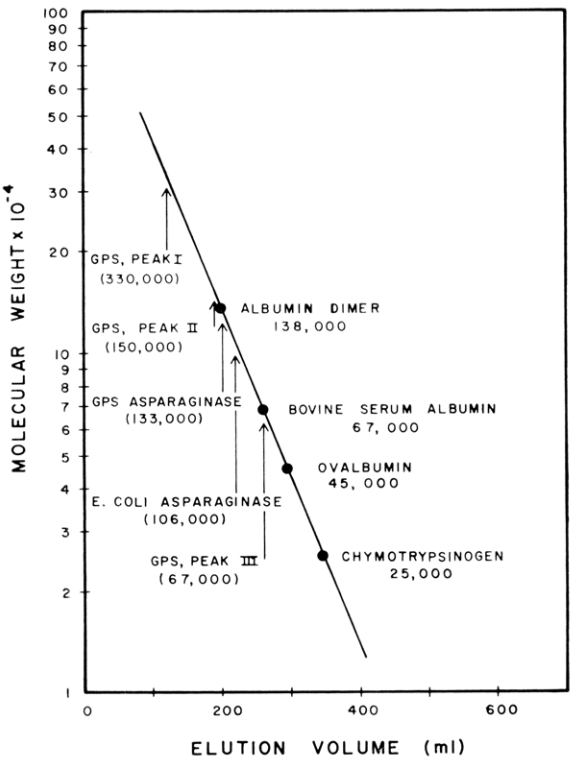

FIGURE 7: Molecular weight determination by gel filtration. A Sephadex G-200 column, 2.2 × 100 cm, was equilibrated with 0.05 M Tris–HCl buffer, pH 8.0, also 1 M in NaCl. Proteins used for calibration (solid circles) are listed to the right of the plot of molecular weight *vs.* elution volume, and the elution volume of test proteins is shown by arrows. See text for details.

TABLE II: Stability of GPS Asparaginase.[a]

Addn	Incubation Flask	Shaking	% Act.
None	Glass	+	12
BSA, 25 μg/ml	Glass	+	100
Mineral oil, 0.5 ml	Glass	+	69
None	Glass	−	69
None	Polyethylene	+	40

[a] All experiments carried out as described in the Experimental Section under Asparaginase Assay, by incubating for 30 min at 37°.

ments, in excellent agreement with the apparent weight-average molecular weight.

The results of a sedimentation velocity experiment are shown in Figure 6. The enzyme used in this experiment was not homogeneous by immunoelectrophoresis, showing two minor components in the albumin region in addition to the main β-globulin (asparaginase) band. The schlieren pattern, however, indicates a single very pure component. The pattern was photographed at 8-min intervals, and a plot of the log of the distance of the peak from the center of rotation *vs.* time gave a straight line. The sedimentation coefficient was calculated from the slope, and was found to be 6.5 S after correction for solvent viscosity and temperature (Schachman, 1957).

The molecular weight of GPS asparaginase was also determined by measuring the elution volume on a column of G-200 Sephadex. Proteins listed to the right of the curve in Figure 7 were used as standards, with the molecular weights as indicated. The molecular weights shown in parentheses under the proteins listed on the left of the curve were estimated from the standard curve. GPS asparaginase was found to have a molecular weight of 133,000. If the standard curve is constructed using a molecular weight of 69,000 for albumin, then the apparent value for the molecular weight of GPS asparaginase is even closer to that determined by sedimentation equilibrium (137,000 and 138,000, respectively). The molecular weight of asparaginase from *E. coli* B (Mashburn and Wriston, 1964) was also determined, and found to be 106,000, significantly lower than the GPS enzyme.

TABLE III: Amino Acid Composition of GPS L-Asparaginase.

Amino Acid	mμmoles 22 hr	mμmoles 72 hr	Apparent Value	Amino Acid/Asp	Minimum No. of Residues	Residues/ 140,000 mol wt
Asp	31.8	31.8	31.8	1.000	10	90
Thr	28.4	26.6	29.2	0.918	9	81
Ser	36.9	30.9	39.5	1.242	12	108
Glu	38.3	39.3	38.8	1.220	12	108
Pro	30.6	33.0	31.8	1.000	10	90
Gly	30.2	30.5	30.4	0.956	10	90
Ala	24.7	25.1	24.9	0.783	8	72
Val	29.3	32.6	32.6	1.025	10	90
Half-Cyst	7 0	6.5	7.0	0.220	2	18
Met	4.0	1.4	4.0	0.126	1	9
Ileu	14.3	15.9	15.9	0.500	5	45
Leu	43.1	44.2	44.2	1.390	14	116
Tyr	5.5	5.1	5.5	0.173	2	18
Phe	11.4	12.4	12.4	0.390	4	36
Lys	18.4	18.7	18.6	0.584	6	54
His	8.0	7.9	8.0	0.252	3	27
Arg	16.1	16.3	16.2	0.509	5	45
Try	...	...	...	...	1	9

Amino Acid Analyses. An aliquot of the same enzyme preparation used in the sedimentation equilibrium experiment was subjected to amino acid analysis (see Methods) and the results are shown in Table III. The minimum molecular weight calculated from these data is 15,539. Multiplication of this value by nine gives a molecular weight of 139,850, which is within 1% of the value obtained by sedimentation equilibrium ultracentrifugation.

Discussion

Asparaginase activity is widely distributed in nature, having been found in animal tissue, plants, and microorganisms (Zittle, 1951; Kretovich, 1958). Asparaginase activity was discovered in guinea pig serum by Clementi (1922) and, as mentioned in the introduction, partial purification of this enzyme has been reported by several groups of investigators. The purification procedures described here have led to a preparation which appears to be homogeneous on the basis of several different criteria: (1) equilibrium sedimentation by the method of Yphantis (1964); (2) migration of a single symmetrical peak during velocity sedimentation; (3) the appearance of a single arc upon immunoelectrophoresis; (4) a single band in polyacrylamide gel electrophoresis (Yellin and Wriston, 1966); and (5) the amino acid analytical results are compatible with a pure protein with a molecular weight of *ca.* 138,000.

Broome (1961, 1963) was the first to present evidence that asparaginase was the substance in guinea pig serum which was responsible for its antilymphoma effect, and we have recently presented evidence (Yellin and Wriston, 1966) confirming this, using the highly purified GPS asparaginase described in this paper. We have also found that asparaginase from *E. coli* B has antilymphoma activity (Mashburn and Wriston, 1964) and have now compared certain properties of the two enzymes. The molecular weight of *E. coli* asparaginase (gel filtration, Figure 7) was found to be 106,000 as compared to 133,000 by this method for the GPS enzyme. The *E. coli* enzyme is completely soluble at half-saturation with sodium sulfate (15%) whereas the GPS enzyme is precipitated. The *E. coli* enzyme is strongly adsorbed on calcium hydroxylapatite columns in 0.03 M potassium phosphate buffer, pH 6.8, whereas the GPS enzyme is only slightly retarded, but the two enzymes would appear to have similar isoelectric points since they are eluted from a DEAE column at approximately the same position (Yellin and Wriston, unpublished). The *E. coli* enzyme is more effective as an antilymphoma agent on a unit for unit basis (Mashburn and Wriston, 1964).

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References

Andrews, P. (1964), *Biochem. J. 91*, 222.

Avizonis, P. V., and Wriston, J. C., Jr. (1962), *Anal. Chem. 34*, 54.

Beaven, G. H., and Holiday, E. R. (1952), *Advan. Protein Chem. 7*, 375.
Boyer, S. H., Fainer, D. C., and Naughton, M. A. (1963), *Science 140*, 1228.
Broome, J. D. (1961), *Nature 191*, 1114.
Broome, J. D. (1963), *J. Exptl. Med. 118*, 99, 121.
Campbell, D. H., Garvey, J. S., Cremer, N. G., and Sussdorf, D. H. (1963), Methods in Immunology, New York, N. Y., Benjamin, p 143.
Clementi, A. (1922), *Arch. Intern. Physiol. 19*, 369.
Everall, P. H., and Wright, G. H. (1958), *J. Med. Lab. Tech. (London) 15*, 209.
Fahey, J. L., and Horbett, A. P. (1959), *J. Biol. Chem. 234*, 2645.
Filmer, D. L., and Koshland, D. E., Jr. (1963), *Biochim. Biophys. Acta 77*, 334.
Flodin, P., and Killander, J. (1962), *Biochim. Biophys. Acta 63*, 403.
Hamilton, P. B. (1962), *Ann. N. Y. Acad. Sci. 102*, 55.
Hamilton, P. B. (1963), *Anal. Chem. 35*, 2055.
Hamilton, P. B. (1965), *Nature 205*, 284.
Hjerten, S. (1959), *Biochim. Biophys. Acta 31*, 216.
Kretovich, W. L. (1958), *Advan. Enzymol. 20*, 319.
Levin, O. (1962), *Methods Enzymol. 5*, 27.
Mardashev, S. R., and Shao-Khua, V. (1962), *Dokl. Akad. Nauk SSSR 142*, 709.
Mashburn, L. T., and Wriston, J. C., Jr. (1963), *Biochem. Biophys. Res. Commun. 12*, 50.
Mashburn, L. T., and Wriston, J. C., Jr. (1964), *Arch. Biochem. Biophys. 105*, 451.
Meister, A. (1955), *Methods Enzymol. 2*, 383.
Moore, S., and Stein, W. H. (1963), *Methods Enzymol. 6*, 819.
Peterson, E. A., and Sober, H. A. (1962), *Methods Enzymol. 5*, 3.
Raymond, J. (1962), *Clin. Chem. 8*, 455.
Schachman, H. K. (1957), *Methods Enzymol. 4*, 32.
Suld, H. M., and Herbut, P. A. (1965), *J. Biol. Chem. 240*, 2234.
Tiselius, A., Hjerten, S., and Levin, O. (1956), *Arch. Biochem. Biophys. 65*, 132.
Tower, D. B., Peters, E. L., and Curtis, W. C. (1963), *J. Biol. Chem. 238*, 983.
Waddell, W. J. (1956), *J. Lab. Clin. Med. 48*, 311.
Yellin, T. O., and Wriston, J. C., Jr. (1966), *Science* (in press).
Yphantis, D. A. (1964), *Biochemistry 3*, 297.
Zittle, C. A. (1951), *Enzymes 1*, 922.

The Ribonucleic Acid Content of Turnip Yellow Mosaic Virus*

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ABSTRACT: The ribonucleic acid (RNA) content of turnip yellow mosaic virus (TYMV) was derived from an experimentally determined ratio of N:P (21 determinations), and the theoretical nitrogen and phosphorus contents of TYMV–RNA and TYMV–protein which were calculated from the respective base ratio and amino acid composition. The ratio of N:P was 4.64 (σ 0.14), the content of RNA (in the acid form) 33.5%. The nitrogen and phosphorus contents of TYMV were subsequently calculated to be 15.19 and 3.27%, respectively. By relating ultraviolet absorbancy at 260 mμ and the individual nitrogen and phosphorus determinations to each other, and adjusting the latter to the theoretical values, an extinction coefficient of $E_{260}^{1\%} = 86$ was obtained for TYMV. The data are consistent with an optical determination of the RNA content, with a number of recently determined physical constants of TYMV and its constituent components, and with the notion that its capsid is an icosahedral arrangement of 180 protein subunits of 20,000 mol wt.

The detailed structural analysis of viruses, consisting predominantly of nucleic acid and protein, requires an exact knowledge of the absolute amounts in which these components occur in a virus particle. Such knowledge is provided by the determination of the nucleic acid content (usually by chemical means) and of the particle weight (usually by physicochemical methods) of the virus.

Turnip yellow mosaic virus,[1] a small isometric plant virus, has been known to contain a relatively large amount of nucleic acid since it was first purified, although the initial estimates were too low because of the presence of top component particles or empty capsids in the preparations (Markham and Smith,

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[1] Abbreviations used: TYMV, turnip yellow mosaic virus; RNA, ribonucleic acid.