Molecular Weight of Encephalitogenic Protein by Electrophoresis in Sodium Dodecyl Sulfate (SDS)-Acrylamide Gels[1] (36118)

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Molecular weight determinations of the myelin basic protein (BP) which causes experimental allergic encephalomyelitis (EAE) in animals have been reported by several investigators (1–9). Methods employed have included ultracentrifugation (1–3, 9), gel filtration (4, 6, 8), and amino acid analysis (5, 7). Those findings suggest that the molecular weight of BP isolated from chloroform–methanol-extracted brain is approximately 18,000 to 20,000 daltons.

We now report molecular weight estimations of BP obtained by electrophoresis in acrylamide gel following treatment of the protein with sodium dodecyl sulfate (SDS). This method, which was originally described by Shapiro *et al.* (10), is both rapid and simple to perform, and has yielded reliable and reproducible results for a wide variety of proteins (10-12). SDS, an anionic detergent, presumably complexes with proteins to render them anionic, thus minimizing native charge differences. Electrophoretic migration then becomes a function of molecular weight, dependent on the seiving effect of the gel (10–12).

Our findings, which are in reasonable agreement with results obtained from more precise physiochemical measurements, suggest that SDS-acrylamide electrophoresis can be employed as a simple technique to estimate the molecular weight of BP.

Materials and Methods. Proteins. Crystalline bovine serum albumin, ovalbumin (2× crystallized), chymotrypsinogen A (6× crystallized), sperm whale myoglobin, and cytochrome *c* were obtained from Mann Research Laboratories and employed as molecular weight markers. BP was prepared from lyophilized or homogenized fresh bovine brain, by acid extraction at either pH 3.0 or 2.0, following chloroform–methanol (2:1) extraction, as previously described (13–15). Benzyl-BP was prepared by modification of BP with 2-hydroxy-5-nitrobenzyl bromide, as previously described (15).

Preparation of gels. The gel buffer contained 7.8 g of $NaH_2PO_4 \cdot 2H_2O$, 38.6 g of $Na_2HPO_4 \cdot 7H_2O$ and 2 g of SDS/liter (12). Ten percent acrylamide solution was prepared by dissolving 22.2 g of acrylamide and 0.6 g of methylene bisacrylamide in 100 ml of distilled water, filtered through Whatman No. 1 filter paper and stored at 4° in a dark bottle (12). Fifteen milliliters of gel buffer was deaerated, mixed with 13.5 ml of acrylamide solution, and deaerated a second time. Then 1.5 ml of freshly prepared ammonium persulfate (30 mg/ml) and 0.3 ml of *N,N,N′,N′*,-tetramethylethylenediamine (TEMED) were added and the solution was mixed. Gel columns (6 cm) were prepared in glass tubes (5 mm i.d.), and overlaid with distilled water.

Preparation of samples for electrophoresis. The proteins were dissolved at a concentration of 1 to 2 mg/ml in an incubation solution consisting of 0.4% SDS (10), 0.2% 2-mercaptoethanol, and 4 *M* urea (v/v) (11). This was incubated for 30 to 60 min in a 45° water bath. After incubation, 0.02 ml of bromophenol blue tracer dye (0.05%) was

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added to each incubation mixture, and 0.03 ml of the incubation solution, usually containing 6 μg of protein, was applied to each gel. Proteins were run in duplicate, and each experiment was repeated at least twice.

Electrophoresis. Electrophoresis was carried out in a Buchler Polyanalyst electrophoresis apparatus for 4 hr at 8 mA/gel, with migration toward the anode. The chamber buffer was gel buffer diluted (v/v) with distilled water. After electrophoresis, the SDS was leached out and protein fixed by soaking the gels in 20% sulfosalicylic acid for 18 to 24 hr (11). Gels were then stained for 4 to 6 hr with Coomassie blue (0.02%) freshly prepared in 12.5% trichloroacetic acid (TCA), and destained in 10% TCA (11).

Calculations. The migration distance of each protein was measured, and the conversion to relative mobility was determined by dividing the migration distance by that of chymotrypsinogen A (11). Migration distance and/or relative mobility was plotted against log of molecular weight (10–12).

Results. SDS-acrylamide electrophoresis analyses were carried out on eight different bovine BP preparations. Samples were run in duplicate, and each experiment was repeated two to six times. Usually 6 μg of protein were applied to each gel, although a few experiments were conducted at 3 μg, and one at 60 μg/gel. These variations in concentration did not appreciably alter the mobility, except that 60 μg samples produced very diffuse bands. However, in the latter case, molecular weights could be accurately determined by measuring the leading edge of the band. Trial studies comparing 0.4% and 1% SDS in the incubation mixture (10) revealed no differences, and the lower concentration was employed subsequently for ease of handling.

The results of one experiment are illustrated in Fig. 1. The migration distance of each molecular weight marker protein was measured, and relative mobility was calculated. The plot of relative mobility vs the known molecular weight[2] was linear. Gels containing BP showed one dense major component. When the relative mobility of this protein was determined and fitted into the curve, the value obtained corresponded to a molecular weight of 21,500 daltons (Fig. 1). In the series of experiments, the relative mobility of the major component of bovine BP was found to be 1.10 ± 0.02 [standard deviation (SD)] with respect to chymotrypsinogen A. The molecular weight was subsequently calculated to be 22,166 ± 577 (SD), as determined from relative mobility. When the molecular weight of this BP component was cal-

[2] The molecular weight values of the marker proteins were obtained from Dunker and Rueckert [Ref. (11)].

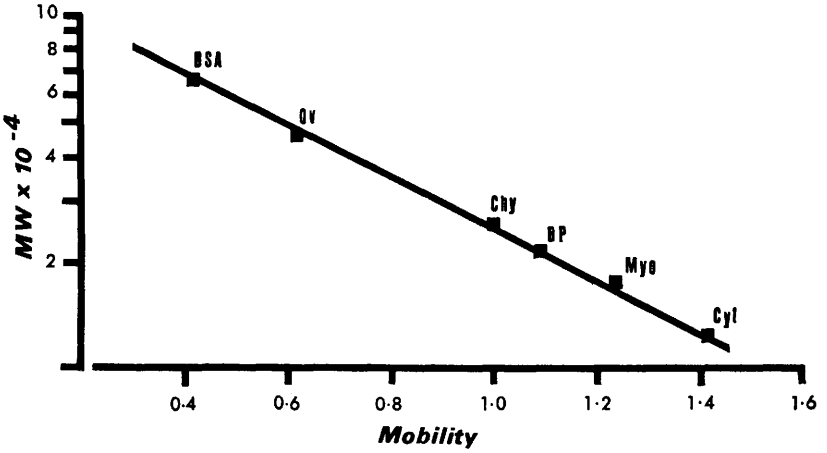

FIG. 1. Plot of the logarithm of the molecular weights versus relative mobilities of bovine serum albumin (BSA, mol wt 66,000), ovalbumin (Ov, mol wt 46,000), chymotrypsinogen A (Chy, mol wt 25,741), BP, myoglobin (Myo, mol wt 17,600) and cytochrome *c* (Cyt, mol wt 12,400). In this experiment, the molecular weight of BP was calculated as 21,500 daltons.

culated from the plot of migration distance vs log of molecular weight, the value obtained was 21,894 ± 792 daltons.

In addition to the characteristic major protein component, one or more additional faint bands were observed in some preparations. These were more frequently seen, and more intense, in BP preparations extracted at pH 2.0. Usually these trace components migrated faster than the major protein band, and the most frequently observed corresponded to a molecular weight of 15,000 to 16,000 daltons. This component was also present in some BP preparations extracted at pH 3.0, but was not detected in other preparations which were encephalitogenic at 20 to 30 μg in guinea pigs.

Benzyl-BP, prepared from two of the BP preparations, was identical to the unmodified BP when subjected to SDS-acrylamide electrophoresis.

Discussion. The molecular weights of numerous proteins have been accurately determined following treatment with SDS, and reduction with 2-mercaptoethanol in the presence of urea (10–12). The electrophoretic mobility of proteins treated in this manner is inversely proportional to the log of their molecular weights.

In the present studies, five molecular weight marker proteins and bovine brain BP were treated with SDS in the presence of 2-mercaptoethanol and urea, and subjected to electrophoresis in 10% acrylamide gels containing SDS. With respect to the marker proteins, the plot of migration distance or relative mobility vs log of molecular weight, was linear. The molecular weight of bovine BP was found to be 22,166 ± 577 and 21,894 ± 792, when relative mobility or migration distance, respectively, was plotted against log of molecular weight. These findings, which were consistent for the eight BP preparations studied, are in reasonable agreement with values of about 18,000 to 21,000 daltons obtained by ultracentrifugation (1–3), amino acid composition (5, 7), and gel filtration (6). With respect to molecular weight estimates of BP by gel filtration, Deibler *et al.* (4) reported that the molecular weight markers employed must have the same physicochemical properties as BP. Failure to consider this factor could result in abnormally high molecular weight values for basic protein (4).

In contrast to molecular weight determinations by gel filtration, the SDS-acrylamide electrophoresis method is not influenced by the properties of the proteins under study (10–12). Basic proteins, like cytochrome *c* and lysozyme, do not exhibit anomalous behavior which might be attributed to conformational or charge effects (11). Indeed, Dunker and Rueckert (11) have shown, using substituted lysozyme bearing charged substituents, that treatment with SDS eliminates even large differences in intrinsic charge. In the present study, cytochrome *c* was employed as a molecular weight marker, and migrated as expected for a protein of its molecular size. However, our molecular weight estimates of BP tended to be somewhat high, suggesting an interaction between this cationic protein and the gel.

An additional BP preparation, which was inadvertently left at room temperature overnight prior to extraction with chloroform–methanol, failed to exhibit the characteristic component. Instead, a diffuse, rapidly migrating constituent with a molecular weight of 8,000 to 9,000, which probably represented brain cathepsin-digested BP (8, 9), was observed. This BP preparation was encephalitogenic in guinea pigs.

The increased heterogeneity of BP extracted at pH 2.0 is in agreement with previous reports that, at lower pH, more extraneous basic proteins are solubilized (13).

Tryptophan-modified BP (benzyl-BP) was identical to unmodified BP. This alteration results in masking of the site responsible for EAE in guinea pigs (15, 17) but not Lewis rats (16) or rabbits (17).

It has recently been reported (18) that the molecular weight of human BP and the large rat BP molecule is 18,000 to 19,000, as determined by SDS-acrylamide electrophoresis.

Summary. SDS-acrylamide electrophoresis studies indicate that the molecular weight of the basic encephalitogenic protein isolated from chloroform–methanol-extracted bovine

brain is 22, 166 ± 577 daltons.

1. Chao, L.-P., and Einstein, E. R., J. Neurochem. **17**, 1121 (1970).

2. Oshiro, Y., and Eylar, E. H., Arch. Biochem. Biophys. **138**, 606 (1970).

3. Tomasi, L. G., and Kornguth, S. E., J. Biol. Chem. **242**, 4933 (1967).

4. Deibler, G. E., Martenson, R. E., and Kies, M. W., Biochim. Biophys. Acta **200**, 342 (1970).

5. Eylar, E. H., Proc. Nat. Acad. Sci. U.S.A. **67**, 1425 (1970).

6. Chao, L.-P., and Einstein, E. R., J. Chromatogr. **42**, 485 (1969).

7. Carnegie, P. R., Nature (London) **229**, 25 (1971).

8. Kies, M. W., and Alvord, E. C., Jr., *in* "Pathogenesis and Etiology of Demyelinating Diseases" (K. Burdzy and P. Kallos, eds.), p. 182. Karger, Basel (1969).

9. Chao, L.-P., and Einstein, E. R., J. Biol. Chem. **243**, 6050 (1968).

10. Shapiro, A. L., Vinuela, E., and Maizel, J. V., Jr., Biochem. Biophys. Res. Commun. **28**, 815 (1967).

11. Dunker, A. K., and Rueckert, R. R., J. Biol. Chem. **244**, 5074 (1969).

12. Weber, K., and Osborn, M., J. Biol. Chem. **244**, 4406 (1969).

13. Kies, M. W., Annals N.Y. Acad. Sci. **122**, 161 (1965).

14. Swanborg, R. H., J. Immunol. **102**, 381 (1969).

15. Swanborg, R. H., J. Immunol. **105**, 865 (1970).

16. Swanborg, R. H., and Amesse, L. S., J. Immunol. **107**, 281 (1971).

17. Chao, L.-P, and Einstein, E. R., J. Biol. Chem. **245**, 6397 (1970).

18. Eng, L. F., Fed. Proc., **30**, 1248 Abstr. (1971).

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