MOLECULAR WEIGHT ESTIMATION OF APOFERRITIN SUB-UNITS

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1. Introduction

A technique has recently been devised [1–4] for the estimation of the molecular weight of proteins and polypeptide chains by an empirical approach based on the determination of the rate of migration on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS). The reliability of the method has been demonstrated by Weber and Osborn [5] who studied forty proteins with well characterised polypeptide chains, and showed that in no case did the technique give results which were in disagreement with generally accepted values obtained by other methods.

In the present communication we describe the application of this procedure to the determination of the molecular weight of the sub-unit of horse spleen apoferritin. The results obtained lead to a value for the molecular weight of the sub-unit which is in disagreement with previously determined values, and this observations is discussed in relation to the number of sub-units present in the apoferritin molecule and to previously determined values for the molecular weight of undissociated apoferritin.

2. Materials and methods

Albumin, apofferitin (horse spleen), catalase, chymotrypsinogen, ferritin (horse spleen), γ-globulin and ovalbumin were obtained from Mann Research Laboratories; chymotrypsin, trypsin, papain and leucine amino peptidase from Worthington Biochemical Corporation; cytochrome *c*, pepsin and ribonuclease from Sigma Chemical Company and myoglobin from Seravac Laboratories Ltd. Apoferritin from horse spleen was also prepared from ferritin by the method of Granick and Michaelis [6] as modified by Crichton [7].

The method of electrophoresis employed was essentially that described by Weber and Osborn [5] except that the gel tubes were longer (13.0 cm) and that the protein bands were stained with amido black. Scanning of the gels was carried out using the Vitatron densitometer.

3. Results and discussion

The proteins used in this study as molecular weight markers together with their molecular weights are listed in table 1.

Since the gels expanded slightly after staining and destaining the rate of migration was corrected as follows:

$$\text{electrophoretic mobility} = \frac{\text{distance of protein migration} \times \text{gel length before staining}}{\text{distance of dye migration} \times \text{gel length after staining}}$$

When the electrophoretic mobilities of these markers were plotted against the logarithm of molecular weight of the polypeptide chains, a linear relationship was obtained (fig. 1).

The proteins were prepared for electrophoresis by incubation in 0.01 M sodium phosphate buffer, 1% SDS, 1% 2-mercaptoethanol, pH 7.0 at 37° for 2 hr. After 12 hr dialysis in 0.01 M sodium phosphate buffer, 0.1% SDS, 0.1% 2-mercaptoethanol, pH 7.0 at room temperature, samples were applied to the gels, either individually or mixed, and electrophoresed at 20 ma per gel for about 4 hours (fig. 2).

Table 1

Protein	Molecular weight	Reference
Serum Albumin	68,000	8
Catalase	60,000	9
γ-globulin, H chain	55,000	3
Leucine amino peptidase	53,000	2
Ovalbumin	43,000	10
Pepsin	35,000	11
Chymotrypsinogen	25,700	12
γ-globulin, L chain	25,000	3
Trypsin	23,300	12
Papain	23,000	12
Myoglobin	17,200	12
Ribonuclease	13,700	12
Chymotrypsin, H chain	13,000	12
Cytochrome *c*	11,700	12
Chymotrypsin, L chain	11,000	12

Proteins used as molecular weight markers in SDS-electrophoresis.

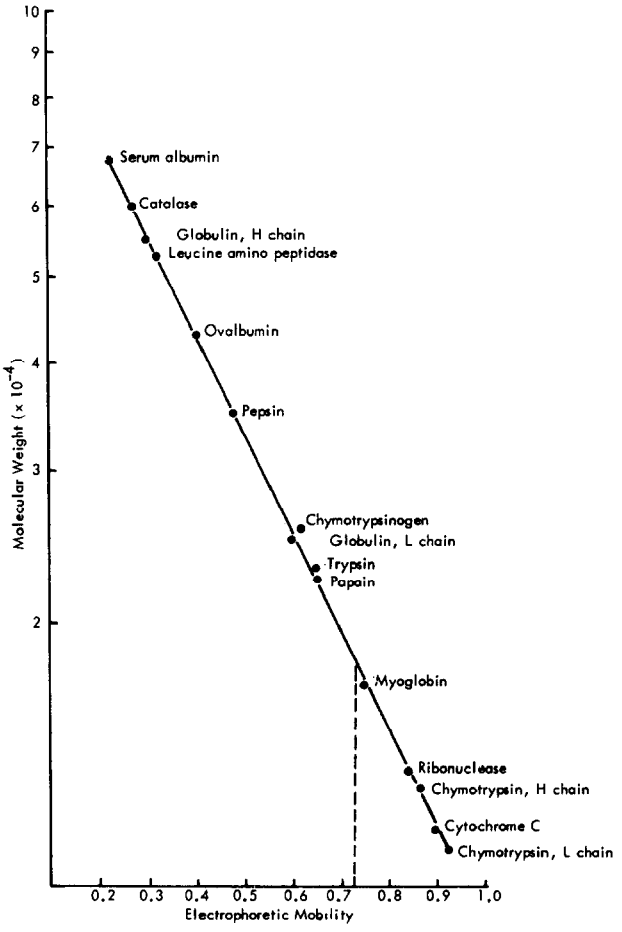

Fig. 1. The electrophoretic mobility of the marker proteins is shown as a function of their respective molecular weights in the presence of sodium dodecyl sulphate. The broken line indicates the experimentally determined electrophoretic mobility for the ferritin and apoferritin sub-unit and this corres ponds to a value of 18,200 ± 1,100 for the molecular weight.

From this treatment, the average electrophoretic mobility of dissociated apoferritin was calculated to be 0.732 ± 0.022 and this corresponds to a polypeptide chain of molecular weight 18,200 ± 1,100.

Ferritin was treated similarly and this gave an amido black positive band with an average electrophoretic mobility of 0.730 ± 0.022 which is also equivalent to a molecular weight of 18,200 ± 1,100. A narrow band which contained iron was observed to travel a very short distance into the gel (electrophoretic mobility = 0.038).

In view of the earlier results of Hofmann and Harrison [13] the fact that both apoferritin and ferritin appear to dissociate into sub-units in the presence of SDS is of considerable interest. They found that SDS/protein ratios of greater than 1:3 (w/w) were required for dissociation of apoferritin. The dissociation of ferritin into sub-units by this method, together with the possibility of spontaneous reassociation of apoferritin molecules following extensive dialysis, may provide a rapid and convenient procedure for preparation of apoferritin from ferritin without the need for reduction of the ferric ion.

Hofmann and Harrison [13] obtained a value of 25000 to 27000 for the molecular weight of the apoferritin sub-unit by determining the sedimentation and diffusion coefficients and also by the approach-to-equilibrium method. This is in close agreement with a value of 23000 calculated from the tryptophan content [14, 15] and 24000 calculated from the number of *N*-acetyl residues (19.5 ± 5% acetyl residues/mole apoferritin [16]).

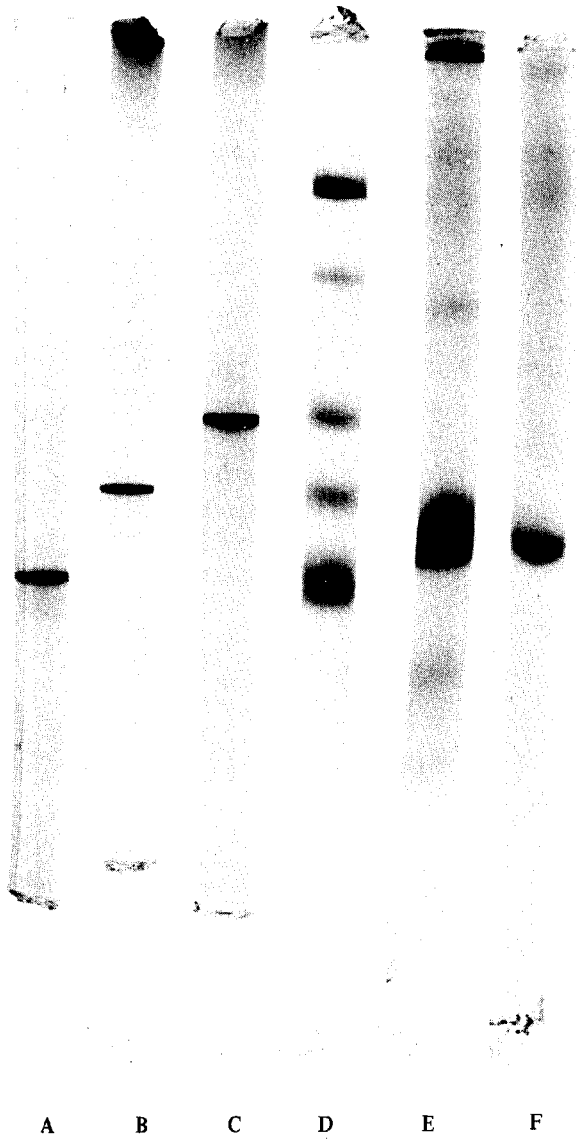

Fig. 2. The protein bands shown in each gel are as follows: (A) myoglobin (17,200), (B) chymotrypsinogen (25,700), (C) pepsin, (35,000), (D) myoglobin (17,200), chymotrypsinogen (25,700), pepsin (35,000), leucine amino peptidase (53,000) and serum albumin (68,000) - the proteins are listed from bottom to top, (E) ferritin, (F) apoferritin. By comparison of the various gels, E and F give some indication of the molecular weight of the ferritin and apoferritin sub-unit but, as they were electrophoresed on a different occasion from A–D, they are not directly comparable.

The value of 18,200 ± 1,100 would lead to a value of 364,000 ± 22,000 for the undissociated protein on the basis of 20 sub-units, which is in poor agreement with previously determined values [16–18] which range from 430,000 to 480,000. However, if there are 24 sub-units per apoferritin molecule we would have a molecular weight for the undissociated protein of 437,000 ± 26,000. This suggests that if the value reported here is correct, the total number of sub-units may well be 24 which is one of the possible values suggested by Harrison et al. [14].

As was indicated earlier, the determination of molecular weights by SDS-polyacrylamide gel electrophoresis is purely empirical and as such it obviates the necessity of calculating the amount of SDS bound to the protein. Another such empirical method recently described is the determination of the molecular weight of polypeptide chains by gel filtration in 6M guanidine hydrochloride [20]. Since it is known that apoferritin does dissociate in the presence of 6M guanidine hydrochloride [21] it has been decided to use this technique in the hope of resolving the conflicting data.

A report of this work will follow.

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