RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND AREA OF PROTEIN FILMS IMMOBILIZED ON PVC MEMBRANES

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1. Introduction

It was shown recently that proteins can be spread and immobilized in homogeneous films with sharp contours on polyvinylchloride (PVC)* membrane filters during chromatographic development in neutral aqueous solutions [1]. The linear relationship between the amount of protein applied and the area covered by the film [1, 2] made it possible to determine proteins quantitatively in amounts of about 1–5 μg in 1–5 μl, within a few minutes. Similar results were achieved also with films of high-molecular polyethylene glycols (PEG)* [3]. Moreover, with the PEG, a linear relationship was found between the logarithm of the molecular weights and the areas of films corresponding to a constant amount of material. A good correlation was observed between the experimental data and the theoretical model [3] based on the assumption that PEG molecules of different molecular weights are adsorbed to PVC in condensed films of the same order.

In the present experiments, we investigated whether an analogous relationship between the molecular weight and the area of the immobilized film could also be found for proteins.

2. Methods

Strips 3–5 mm × 10–30 mm of PVC membrane filters Sartorius (Göttingen, G.F.R.) SM 12801, were wetted in 40% aqueous ethanol, placed on a pile of dry filter papers (Whatman 1) and washed thoroughly with 3–5 ml of an aqueous solution of 0.1 M phosphate buffer, pH 7.2, – 0.9% NaCl (1:1, v/v). Randomly selected standard proteins enumerated in fig. 1 (the same samples as in [2]) were dissolved in the diluted buffer (about 0.2% solutions) and their exact concentrations were checked by dry weight. 0.1, 0.2, or 0.4 μl were applied stepwise by one calibrated capillary onto a polished plexi glass slide and soaked quantitatively into the starting edge of the strip as described in [1, 2]. Ascending chromatographic development was made with the same diluted buffer, pH 7.2, at 23–26° until the flow marker (5% aqueous potassium bichromate or 5% copper chloride) reached the upper end of the strip (usually within 1–3 min). This upper end was held gently between a glass slide and a dry Whatman 1 filter paper wick to ensure a regular upward suction of the buffer from the lower paper wick [1, 2]. The protein layer was stained with 0.5% amido black 10B in 5% trichloroacetic acid and the stained area was measured by using a transparent millimeter scale [2]. An auxiliary straight-line calibration curve was constructed for each sample by plotting the area in mm^2 versus the amount of applied protein in μg [cf. 2–4]. Then the areas corresponding to 4.0 μg of each sample were read and plotted versus the logarithm of M.W. of the given protein (fig. 1).

* *Abbreviations:*
PVC: polyvinylchloride
PEG: polyethylene glycol.

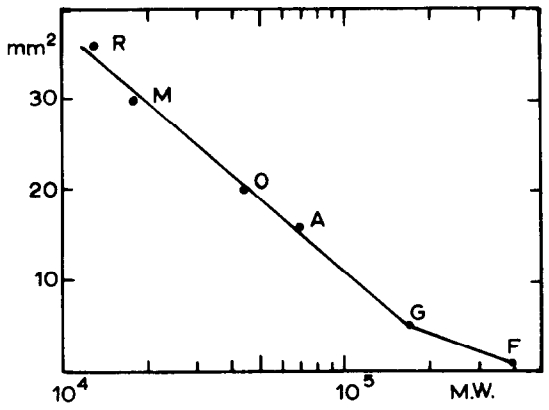

Fig. 1. Relationship between the molecular weight and the area of the protein layer. R: bovine ribonuclease (M.W. 13,000); M: horse myoglobin (M.W. 17,500); O: ovalbumin (M.W. 44,000); A: bovine serum albumin (M.W. 69,000); G: human gamma globulin (M.W. 169,000); F: human fibrinogen (M.W. 400,000). The curve is valid for 4.0 μg of the standard proteins. PVC membrane ultrafilters Sartorius SM 12801 were used for membrane chromatography in the phosphate buffer, pH 7.2. Protein areas were stained with aminoblack 10B. Average values of M.W. were taken from the literature [7].

3. Results and discussion

The relationship presented in a semilogarithmic scale (fig. 1) is nearly linear (with the exception of the values of fibrinogen which are already near to the limiting zero value of the scale) and expresses an exponential function of a hyperbole. The formal similarity between the present results and those achieved recently with PEGs [3] allows us to assume also that molecules of the given proteins were spread and immobilized at the PVC-buffer interphase in regular layers of a constant order. This assumption corresponds generally to the conclusions of earlier investigations of protein films spread on gas-liquid interphase [5, 6].

The smooth calibration curve, good reproducibility of the individual measurements (± 3%, cf. [1–4] and experimental ease, makes the microtechnique described here especially feasible for rapid estimations of the molecular weight of very small amounts of well purified proteins. However, the estimation of molecular weight by this method failed with protein samples which formed layers with two or more zones (e.g. trypsin, cytochrome *c*), or in the presence of some high-molecular contaminants, e.g. polyethylene glycols, neutral detergents "Tween" and impurities of protein character, which may compete with the given protein for the membrane surface.

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