measurements using standard polymers of known molecular weights.

Figure 2 shows the plots of the left side of Eq. 1 *versus* protein concentration for three kinds of proteins. In this case, the constant, k, was estimated to be 6.8×10^{-4} mg·ml^{-1}·mV (average of the values estimated using Dextrans T-20, T-70, and T-500 to be 6.7, 7.1, and 6.5×10^{-4}, respectively). The values of dn/dc of the three Dextrans were assumed to be 0.147 ml/g (*10*). The values for bovine serum albumin and ovalbumin were determined to be 0.191 and 0.184 ml/g, respectively. Ribonuclease A was assumed to have the same value of dn/dc as that of bovine serum albumin. Molecular weights of bovine serum albumin, ovalbumin, and ribonuclease A were thus estimated to be 69,000 (65,400 (*11*)), 44,000 (42,700 (*12*)), and 14,000 (13,800 (*13*)), respectively. These values are in good agreement with those obtained by the sequence determination of amino acid residues shown in the parenthesis. The good performance of the light scattering photometer was thus confirmed, and further measurements were performed in a simpler mode as will be described below.

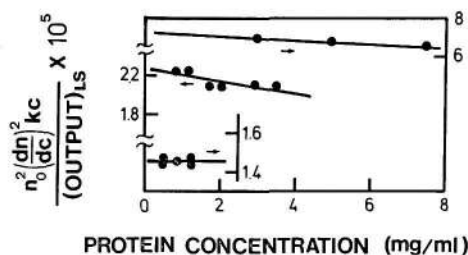

Fig. 2. Determination of molecular weights of the three kinds of proteins by low angle laser light scattering photometer by the batch-type experiments. From top to bottom; ribonuclease A, ovalbumin, and bovine serum albumin. See text for the results obtained.

Elution of sample proteins from a column of TSK-GEL G3000SW was monitored by measurement of the changes in light scattering and refractive index using the low angle laser light scattering photometer and the precision differential refractometer, respectively. A typical example of the records is shown in Fig. 3. The arrows on the left indicate a sample injection. The sample was bovine serum albumin dissolved in the elution solvent (0.01 M sodium acetate of pH 6 containing 0.2 M NaCl and 0.02% NaN_3) to a final concentration of 1.0 mg/ml, and loaded beforehand in the sample loop with an internal volume of 200 μl. The changes in the intensity of light scattering recorded at the bottom appear ahead of the corresponding ones in the refractive index, because of the differences in the positions of the recorder pens and the cells of the detectors along the flow-line.

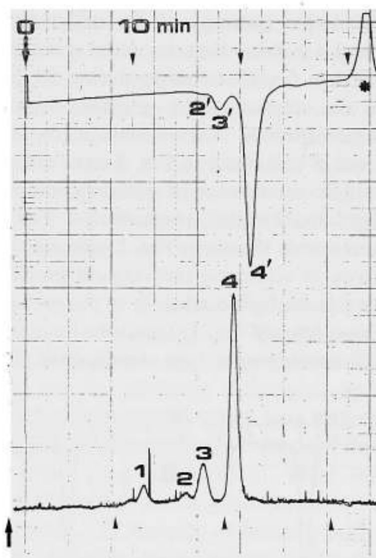

Fig. 3. Elution patterns of bovine serum albumin from the TSK-GEL column detected by measuring changes in refractive index (top) and light scattering (bottom). Sample, 0.20 mg of bovine serum albumin in 200 μl of the elution buffer; chart speed, 30 cm/h; pressure, 30 kg/cm²; flow rate, 0.70 ml/min. Gain setting: LS-8, 16; RI-8, 128. For details, see text.

" Peak 1 " in the record of light scattering seems to correspond to highly aggregated albumin eluted at the void volume. They are negligibly small in quantity as is clear from the absence of a corresponding peak in the record of refractive index. " Peak 2 " in the bottom curve may be assigned to a mixture of oligomers of ablumin, and has a counterpart, " shoulder 2′." " Peak 3 " and the " peak 3′ " may both be safely assigned to the dimer of albumin, because the intensity ratio between them indicates that the component has a molecular weight twice of that of the mono-

meric albumin. The component detected as " peak 4 " and " peak 4′ " is the major component, and can be assigned to the monomeric bovine serum albumin. The trough in the upper curve indicated by the asterisk corresponds to the elution of the solvent of the sample which usually differs slightly from the elution solvent with respect to refractive index.

Ovalbumin and the *A. oryzae* α-amylase were also applied to the measuring system shown in Fig. 1, and gave the elution patterns shown in Fig. 4. Presence of oligomers was observed with ovalbumin (Fig. 4A). Our preparation of the amylase was found to be free from this component (Fig. 4B), and the one used for X-ray analysis also gave a similar pair of elution patterns.

The elution patterns of the major peaks in Figs. 3 and 4 indicate that the initial concentration decreases one fourth or more during its passage through the column at the positions with maximum concentration. Experiments made to draw lines a and a' and line b in Fig. 5 were carried out using sample solutions with protein concentration of 1 mg/ml and 2 mg/ml, respectively. The batch-type experiments shown in Fig. 2 indicate that the dependence of scattering intensity on protein concentration is negligible when it is below 1 mg/ml. The second term of Eq. 1, thus, can be ignored in the gel chromatography-type experiments.

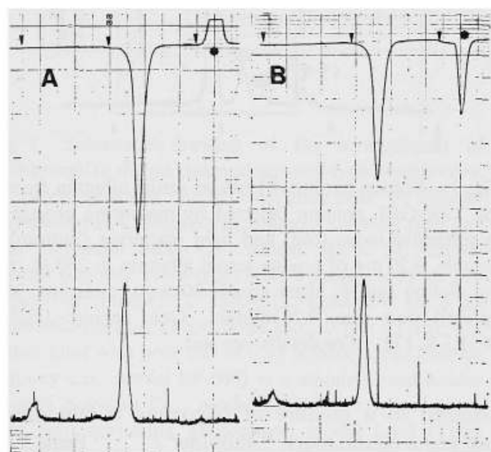

Fig. 4. Elution patterns of ovalbumin (A) and the *A. oryzae* α-amylase (B) obtained in the same manner as in Fig. 3. Sample: A, 0.2 mg; B, 0.14 mg. Only records made 10 min after injection are reproduced.

The output of the refractometer, $(Output)_{RI}$, is nominally said to be proportional to the difference in refractive index between the solution and the solvent. If so, the concentration, c, in Eq. 1 can be replaced by $(Output)_{RI}/(dn/dc)$ with the addition of a dash to the constant, k. Ignoring the second term in Eq. 1, this gives Eq. 2 as:

$$\frac{(Output)_{LS}}{(Output)_{RI}} = n_0^2\left(\frac{dn}{dc}\right)k'M \qquad (2)$$

Strictly speaking, the differential refractometer monitoring the elution must use a light source with the same wavelength as that of the refractometer used to measure dn/dc. The former is actually using a white light. The ratios of the output of the former to that of the latter, therefore, is not necessarily constant among proteins. For the three proteins used in the present chromatography-type experiments, the ratios were, however, found to agree with an error of 2% or less and thus allow the use of Eq. 2 in the present study.

Refractive index increments were estimated to be 0.191, 0.185, and 0.184 ml/g for bovine serum albumin, the α-amylase, and ovalbumin, respectively. Values $(dn/dc)M$ on the right of Eq. 2 were plotted *versus* the output ratios for the standard proteins, bovine serum albumin and ovalbumin, in Fig. 5. The molecular weight of the α-amylase was estimated by interpolation as shown in Fig. 5 for two series of experiments. Line a was obtained using our preparation of the α-amylase. The ratios in the abscissa were estimated from the height of the peaks typical examples of which are shown in Figs. 3 and 4. The two arrows indicate the range of the mean deviation obtained in three experiments. Molecular

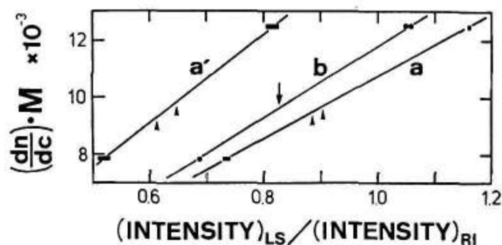

Fig. 5. Calibration lines used to determine molecular weight of the *A. oryzae* α-amylase. For details, see text.