of this solution is measured and the signals due to the referred hydrogen and the exchanging active hydrogen are integrated, the areal intensity-ratio *vs.* the reference signal being determined (β). Then, the number (x) of active hydrogens in the sample molecule can be derived by the following equation.

$$x=(\beta-\alpha)naMs/bMr \quad \text{or} \quad f(\beta-\alpha)naMs/bMr$$

Ms : Molecular weight of the sample molecule.
Mr : Molecular weight of the reference molecule.
n : Number of the reference hydrogens contained in the reference molecule.
α : Ratio in the signal-intensity of the catalysing exchangable hydrogen to the unexchangable reference hydrogen in the blank solvent.
β : Ratio in the signal-intensity of the exchanging hydrogen to the reference hydrogen in the solution containing the sample examined.
f : Factor due to the incompleteness of deuterium exchange. When a large excess of active deuterium is used, it can be approximated by an unit. When not, it depends on the ratio (g) in amount of exchangable hydrogen to that of deuterium in the solution measured. In first approximation, f may be evaluated as (1+g).

The accuracy of this method does depend mainly on that of the instruments for the resonance measurement and the integration. One might expect very accurate results if one could completely neglect the saturation phenomenon, drift of the magnetic field and errors in integrating and recording. Results are shown in the table for examples.

One of the advantages of this method may be the convenient application to the samples soluble only in water, for example, succharides, organic and inorganic salts containing crystal or attached water and so on. A further important application is the quantitative analysis of methyl, methylene and methine hydrogen adjacent to carbonyl functions with very simple procedure as indicated in the table. It might be also promisingly applicable to the kinetic studies. Details will be reported in the full paper.

National Cancer Center, Research Institute, Tsukiji, Chuo-ku, Tokyo

Yutaka Kawazoe (川添 豊)
Masako Ohnishi (大西柾子)

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Light Scattering Titration of Lens Proteins

In order to consider the effect of drugs against cataract, it is necessary to have information on the interaction of the drugs with lens proteins, together with the informations already obtained[1~6] on the penetration of drugs, such as phenoxazone compounds, through the lens capsule.

1) M. Nakagaki, N. Koga, S. Iwata : Yakugaku Zasshi, **82**, 1134 (1962).
2) *Idem* : *Ibid.*, **82**, 1138 (1962).
3) *Idem* : Folia Ophthal. Jap., **13**, 55 (1962).
4) *Idem* : Yakugaku Zasshi, **83**, 275 (1963).
5) *Idem* : *Ibid.*, **83**, 279 (1963).
6) *Idem* : *Ibid.*, **83**, 368 (1963).

Several investigators[7~10] have studied the separation, purification and physical chemical properties of the lens proteins. However, no result has been reported yet on the change of scattered light intensity on the process of precipitation titration of the lens proteins. The results obtained are reported in this paper.

The protein was obtained from the lenses of 3 or 4 years old bovine eyes immediately after slaughter. From the lenses, the cortical region was isolated, and ground in a mortar with distilled water of about four times in weight. The suspension was centrifuged at 10,000 rev./min. for 60 minutes. The supernatant was made up to a solution of 0.3% protein content and used for further experiment. All these processes were proceeded in a cold room at 4°.

Light scattering titration was made with a light scattering apparatus of Brice type*1 equipped with a thermostat. The light intensities at the scattering angles $\theta=0°$, 45°, 90°, and 135° were recorded automatically. The temperature of the solution during the light scattering titration was kept at 5° or 34°, the latter being close to the temperature of living bovine eyes,[6] that is 33.9°. As the precipitant, 0.1% acetic acid was used. The precipitant was added automatically at a constant rate into the protein solution, volume of the latter being kept constant by overflowing the excess solution continuously. The wave length of light used was 436 mμ. At the points of maxima or minima of the scattered light intensity, pH value of the solution in the cell was measured by a glass-electrode pH meter.

The result of the light scattering titration of unfractionated protein from the cortical region is

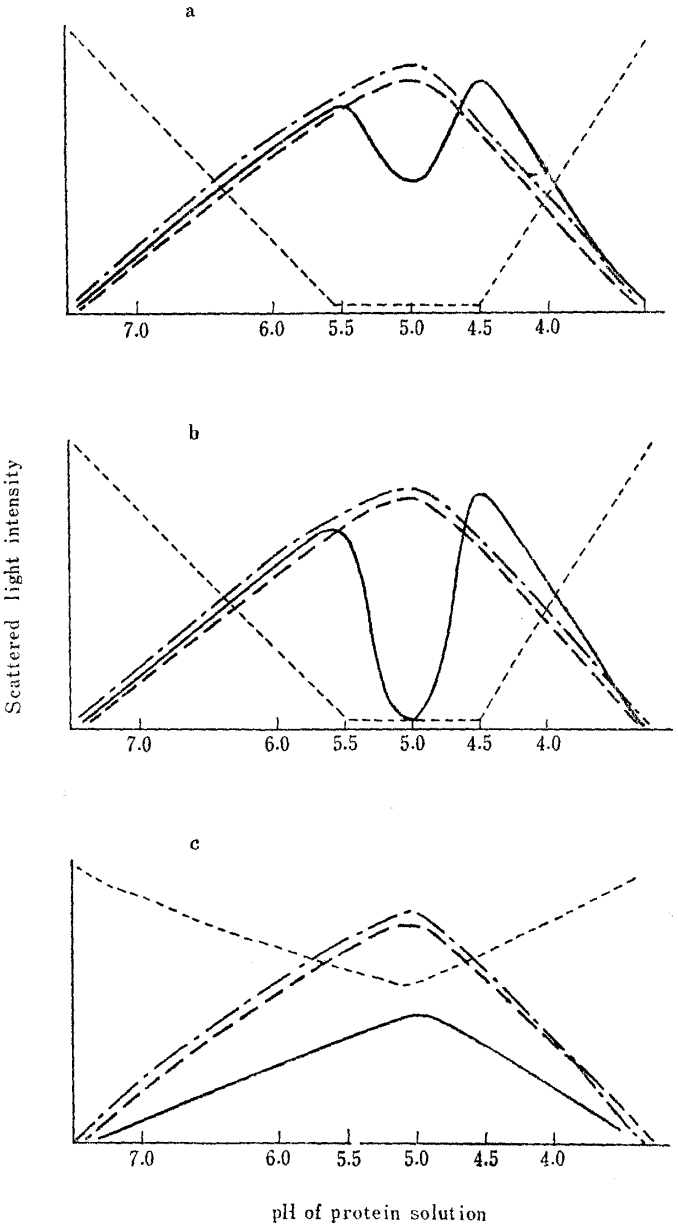

Fig. 1. Light Scattering during Precipitation Titration of Soluble Protein of Lens Cortex with 0.1% Acetic Acid at 5°

a: Unfractionated lens protein
b: Precipitate at pH 5.2 (α-crystallin)
c: Supernatant at pH 5.2 (β-crystallin)
Curves of light intensity at $\theta=0°$(- - - - - - -), 45°(————), 90°(—— • ——), and 135°(— — —).

*1 Shimadzu Photoelectric Light Scattering Photometer, Type PG-21.

7) C. T. Mörner: Z. physiol. Chem., Hoppe-Seyler's, **18**, 61 (1894).
8) A. C. Krause: Arch. Ophthalmol., **8**, 166 (1932).
9) J. Papaconstantinou, R. A. Resnik, E. Saito: Biochim. et Biophys. Acta, **60**, 205 (1962).
10) S. K. Niyogi, V. L. Koenig: *Ibid.*, **69**, 283 (1963).

shown in Fig. 1a. When the pH value became about 5.0, the intensity of transmitted light ($\theta=0°$) reached a minimum point and the intensity of light scattered at $\theta=90°$ and 135° reached a maximum point, respectively. These curves show that the precipitation point (which is very probably equal to the iso-electric point) of the protein is pH 5.0. On the other hand, it is found that the curve for $\theta=45°$ is very characteristic and has two maximum points at pH=5.5 and pH=4.5, and the minimum point between them is at pH=5.0.

The protein was, then, fractionated into two fractions, α-crystallin (precipitate) and β-crystallin (supernatant), by adding 0.1*M* hydrochloric acid as much as to reach pH 5.2, according to the method as used by Francois, *et al.*,[11] and the light scattering titration has been carried out for each fraction. Curves for the light intensity at $\theta=0°$, 90°, and 135° showed one of each maximum point at pH=5.0 for both α-crystallin (Fig. 1b) and β-crystallin (Fig. 1c) as it was for the unfractionated protein (Fig. 1a). As for the curve for $\theta=45°$, α-crystallin showed two maxima and one minimum as the unfractionated protein did, but the light intensity at the minimum point was zero on the contrary to the unfractionated one. On the other hand, β-crystallin showed only one maximum at pH=5.0.

The curve of light scattering titration at $\theta=45°$ for unfractionated protein can, therefore, be explained as the superposition of two curves of α- and β-crystallin. Such a characteristic feature of the curve at $\theta=45°$, probably due to the change of shape of the protein molecules, will be useful for further investigation of the protein of this kind.

Faculty of Pharmaceutical Sciences,
Kyoto University,
Shimo-adachi-cho, Sakyo-ku, Kyoto

Masayuki Nakagaki (中垣正幸)
Naofumi Koga (古賀直文)
Shuzo Iwata (岩田修造)

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Fluorometric Analysis of Pyruvic Acid with 4′-Hydrazino-2-stilbazole

The existing methods for determination of ketones by formation of hydrazones depend on absorption spectroscopy[1~3] and have not sufficient sensitivity for biochemical analysis. In order to establish a more sensitive analytical method, some fluorescent derivatives of hydrazine were investigated.

A reagent specifically selected for determination of α-oxo acids was 4′-hydrazino-2-stilbazole (I) which was synthesized by the route shown in Chart 1. The compound melt at 138° (*Anal.* Calcd. for $C_{15}H_{13}N_3$: C, 73.70; H, 6.16; N, 19.90. Found : C, 73.84; H, 6.11; N, 20.14).

1) M. F. S. El. Hawary, R. H. S. Thompson : Biochem. J., **53**, 340 (1953).
2) O. Seligson, B. Shapiro : Anal. Chem., **24**, 754 (1952).
3) T. E. Friedemann, G. E. Haugen : J. Biol. Chem., **147**, 415 (1943).