was also obtained with the molar ratio of 1:1. From 1.60 g of selenocysteamine hydrochloride and 1.19 g of $CoCl_2 \cdot 6H_2O$ 1.9 g of the complex was obtained.

Cobalt Chelate——Selenocysteamine hydrochloride (1.61 g, 0.01 mole) and NaOH (0.80 g, 0.02 mole) were dissolved in 20 ml of water. This solution was added slowly with stirring to 0.80 g of $CoCl_2 \cdot 6H_2O$ (0.0033 mole) in 20 ml of water. Color of the solution turned to green and deep green crystals separated out after stirring for 6 hr. The product was collected by filtration and washed twice with 10 ml portions of water, twice with 10 ml portions of EtOH, and dried *in vacuo* over P_2O_5, yield 1.2 g.

Polynuclear Cobalt Complex——This complex was prepared almost similarly to the case of polynuclear nickel complex. From 1.60 g of selenocysteamine hydrochloride and 1.81 g of $CoCl_2 \cdot 6H_2O$ 1.60 g of the complex was obtained by the use of dehydrated MeOH as the solvent in the atmosphere of N_2.

Zinc Chelate——Selenocysteamine hydrochloride (1.6 g, 0.01 mole) was dissolved in 6.5 ml of water and neutralized by the addition of an aqueous solution of NaOH (0.40 g, 0.01 mole). To this solution was added 0.81 g (0.01 mole) of zinc oxide, and the resulted suspension was kept at 40° with stirring, and CS_2 (1 ml, 0.015 mole) was added dropwise. After the addition of CS_2, the suspension was stirred for 4 hr. White solid was isolated and washed with water followed by EtOH, and dried *in vacuo* over P_2O_5, yield 1.5 g.

[Chem. Pharm. Bull. 19(6)1272—1275(1971)]

UDC 547.556.33.03 : 547.962.3.09

Spectral Changes of Various Dyes by Bovine Serum Albumin and by Organic Solvents[1]

Ikuo Moriguchi, Shizuo Fushimi and Nobuyoshi Kaneniwa

School of Pharmaceutical Sciences, Showa University[2]

(Received September 17, 1970)

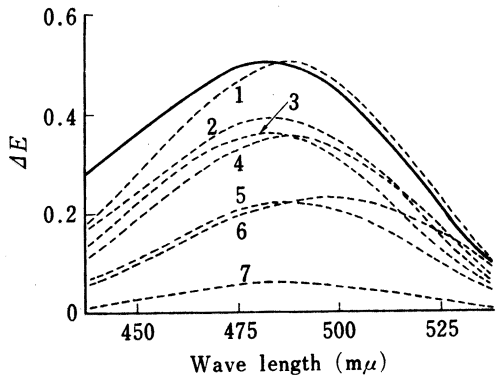

Fig. 1. Spectral Changes of HABA by BSA and by Organic Solvents at 37°

reference: 5×10^{-5}M HABA(Na salt) in water
———: 5×10^{-5}M HABA(Na salt) and 5×10^{-4}M BSA in 0.1M phosphate buffer of pH 7.0
----------: 5×10^{-5}M HABA(Na salt) in 80%(v/v) aqueous organic solvents (1: 2-propanol, 2: dioxane, 3: acetone, 4: 1-propanol, 5: ethanol, 6: hexamethylphosphoramide, 7: methanol)

Protein-induced changes in optical absorption of dyes[3] are well known as metachromasy. In the previous paper[1b] dealing with the mechanism of albumin-induced metachromasy of monoazo dye 2-(4'-hydroxyphenylazo)benzoic acid (HABA), it has been shown that the spectral change of HABA is concerned with the polarity in the dye environment, and that the metachromasy by bovine serum albumin (BSA) can be best simulated when the dye is dissolved in high concentrations of aqueous 2-propanol. In this paper, to see if such a spectral simulation occurs with other dyes, the spectral changes of several kinds of monoazo dyes, diazo dyes, fluoresceins, and sulfonphthaleins by BSA and by organic solvents are investigated.

1) *a*) This forms Part VII of "Spectroscopic Studies on Molecular Interactions"; *b*) Part VI: I. Moriguchi, S. Fushimi, C. Ohshima, and N. Kaneniwa, *Chem. Pharm. Bull.* (Tokyo), **18**, 2447 (1970).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo, 141, Japan.*
3) They are reviewed in I.M. Klotz, "The Proteins," Vol. 1, ed. by H. Neurath and K. Bailey, Academic Press, 1953, p. 727; M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).

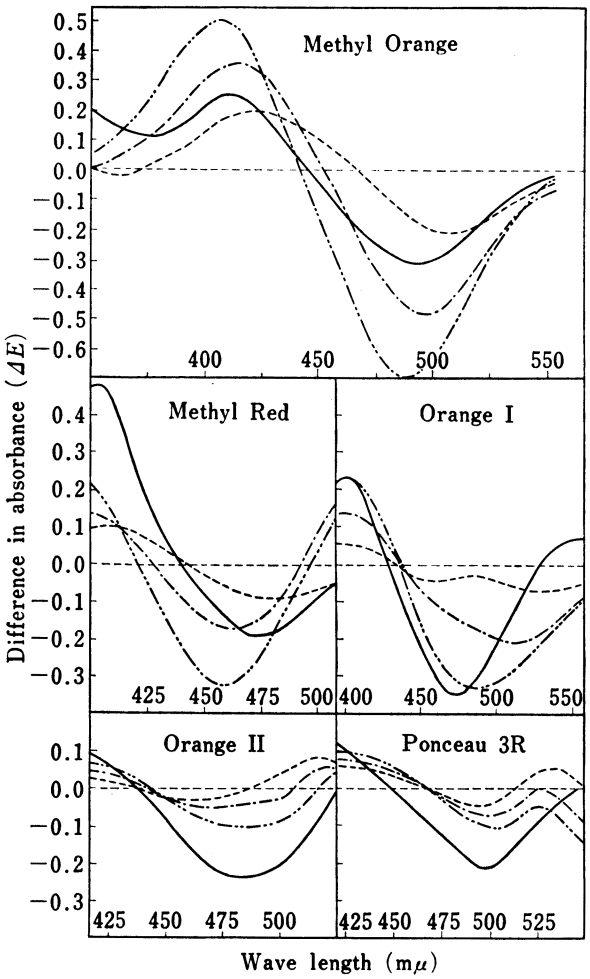

Fig. 2. Spectral Changes of Monoazo Dyes by BSA and by 2-Propanol at 37°

reference: 5×10^{-5}M dye (Na salt) in water
———: 5×10^{-5}M dye (Na salt) and 5×10^{-4}M BSA in 0.1M phosphate buffer of pH 7.0
– – – –: 5×10^{-5}M dye (Na salt) in 30% (v/v) aqueous 2-propanol
———: 5×10^{-5}M dye (Na salt) in 60% (v/v) aqueous 2-propanol
– – – – –: 5×10^{-5}M dye (Na salt) in 90% (v/v) aqueous 2-propanol

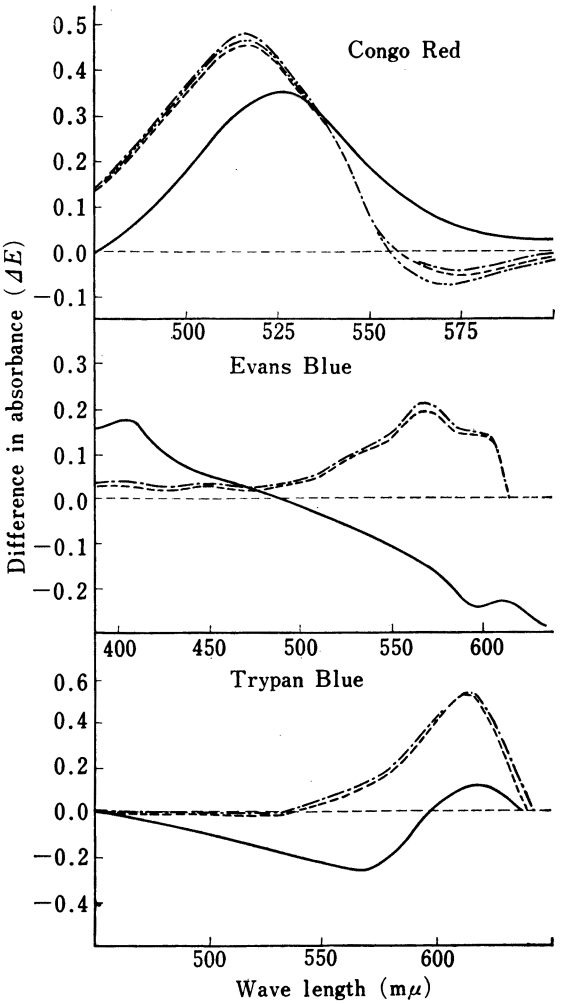

Fig. 3. Spectral Changes of Diazo Dyes by BSA and by 2-Propanol at 37°

reference: 5×10^{-5}M dye (Na salt) in water
———: 5×10^{-5}M dye (Na salt) and 5×10^{-4}M BSA in 0.1M phosphate buffer of pH 7.0
– – – –: 5×10^{-5}M dye (Na salt) in 30% (v/v) aqueous 2-propanol
———: 5×10^{-5}M dye (Na salt) in 60% (v/v) aqueous 2-propanol
– – – – –: 5×10^{-5}M dye (Na salt) in 90% (v/v) aqueous 2-propanol

Experimental

Materials——All the dyes used were of analytical grade from commercial source; orange II from E. Merck AG., methyl orange, methyl red, orange I, congo red, erythrosine, rose bengal, bromcresol green, trypan blue, bromphenol blue, and bromthymol blue from Wako Pure Chemical Industries, Ltd., and evans blue, phenol red, and ponceau 3R from Tokyo Kasei Kogyo Co., Ltd. BSA used was Armour Laboratories Co. "Fraction V." Their purities were checked by absorption spectra, and they were used without further purification.

Measurements of Absorption Spectra——The difference spectra were recorded with a Shimadzu model MPS-50L multipurpose spectrophotometer in 1 cm cells at 37°.

Result and Discussion

The spectral changes (ΔE) of HABA (monosodium salt) by BSA and by 80% (v/v) aqueous organic solvents are shown in Fig. 1, indicating that BSA-induced metachromasy of HABA is reproduced in the presence of organic solvents, especially well of 2-propanol with respect to absorbance. Hence, difference spectra of various dyes in 30, 60, and 90% (v/v)[4] aqueous 2-propanol *vs.* in water were measured to compare with BSA-induced spectral alterations. Five monoazo dyes, three diazo dyes, two fluoresceins, and four sulfonphthaleins are demonstrated in Fig. 2, 3, 4 and 5, respectively. Various degrees of spectral simulation of BSA-induced metachromasy by aqueous 2-propanol are shown with these dyes. The simulation is relatively poor with trypan blue, and is hardly recognized with evans blue. These two dyes, bearing four sulfonate groups in the molecules, seem to be less hydrophobic according to the solubility.[4]

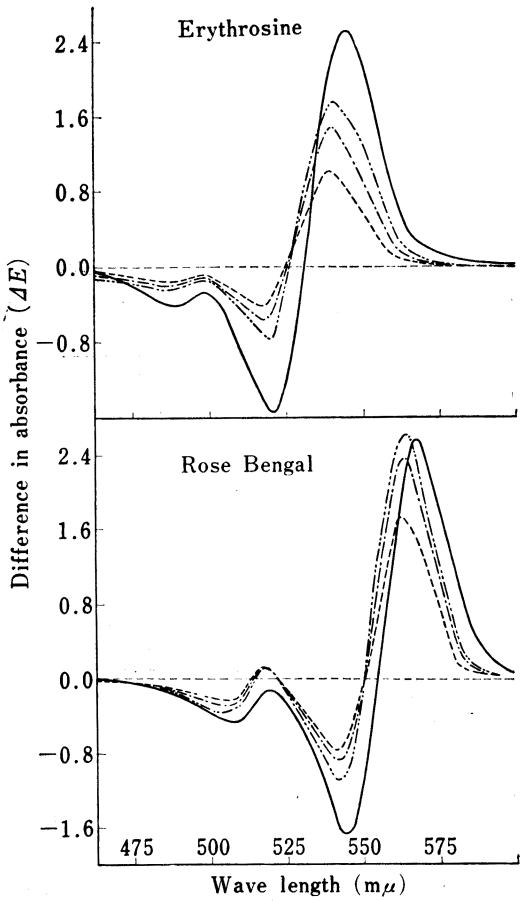

Fig. 4. Spectral Changes of Fluoresceins by BSA and by 2-Propanol at 37°

reference: 5×10^{-6}M dye (Na salt) in water
———: 5×10^{-6}M dye (Na salt) and 5×10^{-4}M BSA in 0.1M phosphate buffer of pH 7.0
———: 5×10^{-6}M dye (Na salt) in 30% (v/v) aqueous 2-propanol
– – – –: 5×10^{-6}M dye (Na salt) in 60% (v/v) aqueous 2-propanol
– – – –: 5×10^{-6}M dye (Na salt) in 90% (v/v) aqueous 2-propanol

It has come to be generally recognized that, in the interior of the protein molecules, non polar side chains provide hydrophobic environments of lower polarity.[5] The interior polarity of BSA molecule has been estimated to be approximately corresponding to that of 2-propanol if the influence of specific intramolecular interactions such as hydrogen bondings on spectral shifts of tryptophan and tyrosine residues is omitted.[1b]

In such circumstance, the above findings suggest that, as well as HABA,[1b] various kinds of dyes bound to BSA may be largely buried in such an environment of lower polarity and, as a result, may more or less change their electronic states which are reflected in the absorption spectra. As is known in the case of 1-dimethylamino-5-sulfonylnaphthalene coupled to BSA,[6] it seems possible and very likely that hydrophobic moieties of bound dyes are buried in the interior of BSA molecules because BSA has high "configurational adaptability"[7] according to Karush.[8]

4) Evans blue and trypan blue were insoluble to 90% (v/v) aqueous 2-propanol.
5) S. Yanari and F.A. Bovey, *J. Biol. Chem.*, **235**, 2818 (1960).
6) M. Laskowski, Jr., *Federation Proc.*, **25**, 20 (1966).
7) It has been recommended to be corrected as "conformational adaptability" (M. Sogami, *Kagaku No Ryoiki*, **23**, 602 (1969)).
8) F. Karush, *J. Am. Chem. Soc.*, **72**, 2705 (1950).

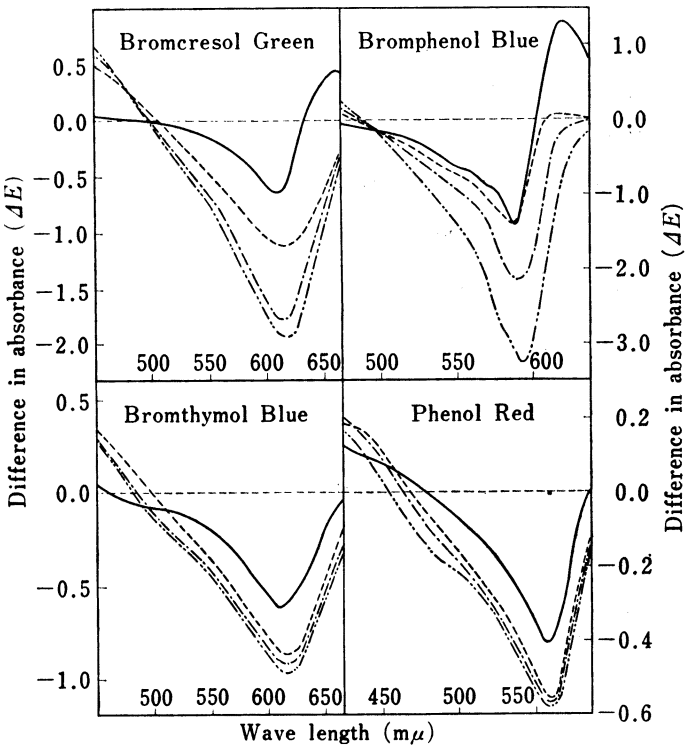

Fig. 5. Spectral Changes of Sulfonphthaleins by BSA and by 2-Propanol at 37°

reference: 5×10^{-5}M dye (Na salt) in water
———: 5×10^{-5}M dye (Na salt) and 5×10^{-4}M BSA in 0.1M phosphate buffer of pH 7.0
———: 5×10^{-5}M dye (Na salt) in 30% (v/v) aqueous 2-propanol
———: 5×10^{-5}M dye (Na salt) in 60% (v/v) aqueous 2-propanol
———: 5×10^{-5}M dye (Na salt) in 90% (v/v) aqueous 2-propanol

Although the spectral simulation of BSA-induced metachromasy by 2-propanol is generally seen with most of the dyes investigated, the diversity of dye molecules in structure, size, hydrophobicity, and number and strength of acidic groups capable of binding to cationic sites on BSA appeares to be reflected in the various shapes of difference spectra presented here.