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Studies on the Interaction of Edible Dyes with Protein I:

Binding parameters of edible dyes with bovine serum albumin

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Abstract The binding of bovine serum albumin (BSA)-edible dyes was studied by spectrophotometric method. The edible dyes used in this study were amaranth, erythrosine, tartrazine and sunset yellow.

The binding free energies and binding sites were determined at pH 7.4. The ranges of edible dye concentration were from 0.3 to 7×10^{-5}M, and those of BSA were from 0.15 to 3×10^{-5}M.

The binding free energies of BSA-edible dyes were from $-6,300$ to $-8,100$ cal/mole.

Keywords Amaranth, Erythrosine, Tartrazine, Sunset yellow, Bovine serum albumin, Klotz method, Binding parameters.

Drug-protein binding has been the subject of extensive research as it relates to the action and fate of specific drugs in the body.

Goldstein[1] and Meyer[2] published a review article about drug-protein binding. They emphasized a potential significance of binding phenomena in the region of toxicological and pharmacological action of drug.

The binding of drug to serum protein, in general, would appear to be undesirable, since drug activity is often suppressed[3-6].

According to the review[7-10], it suggests that hyperkinetic behaviors in children such as restlessness, impulsivity, hyperactivity, short attention span, excitability and oscillating mood can be caused by the ingestion of a large amount of food additives such as food colors and artificial flavors.

Since edible dyes are very widely used and misused in food and drug industry in many countries, it would be very important to investigate the binding of edible dyes to protein.

Various experimental procedures and analysis methods have been used to study drug-protein interaction. These include equilibrium dialysis[11], ultrafiltration[12], gel filtration[13], NMR rates measurements[14], fluorescence techniques[15-16] and UV spectra technique[17-29].

Although the binding free energy obtained by spectrophotometry is generally less than that obtained by dynamic equilibrium, specetrophotometry uses less sample, is operating simply edible dyes are not adsorbed in equilibrium membrane, spectrophotometry is, thus, one of the useful methods in the study of the binding of drugs to protein.

In this paper, we described the binding parameters of BSA-edible dye using this method for the purpose to study the effect of drug addition to BSA-edible dye binding.

EXPERIMENTAL METHODS

Materials

Amaranth, erythrosine, tartrazine and sunset yellow were used as commercial edible dye. Bovine serum albumin was obtained from Sigma Chemical Co. All solutions were prepared in 0.2M-phosphate buffer of pH 7.4.

Apparatus

Absorption spectra measurements were made with Ultraspec 4050 (LKB)-Apple II using 10-mm cell.

Methods

The experimental method was carried out by Klotz method[29].

If molar absorptivity and the concentration of free dye or bound dye at a constant wavelength were ε_1, C_1 or ε_2 and C_2, respectively, the absorbance of the mixed solution is shown as following equation:

$$\log (I_0/I) = \varepsilon_1 C_1 d - \varepsilon_2 C_2 d \quad (1)$$

where d is the thickness of the absorption cell in centimeters.

By using ε_{app}, eq. (1) may be represented as

$$\log (I_0/I) = \varepsilon_{app} (C_1 - C_2) d \quad (2)$$

The fraction of free dye, α, can be shown as follows:

$$\alpha = \frac{C_1}{C_1 + C_2} = \frac{\varepsilon_{app} - \varepsilon_2}{\varepsilon_1 - \varepsilon_2} \quad (3)$$

The ε_1 and ε_{app} were measured from the actual value of the dye solution and dye solution, to which protein was added. Also the values of ε_2 were obtained from the values extrapolated to the intercepts of ε_1 versus $[D]/[P]$ plot, where $[D]$ and $[P]$ are the concentrations of free dye and of total protein in the reaction mixture, respectively.

The number of moles of drug bound per mole of protein, r, the concentration of free dye, C, and the number of binding sites, n were related to the following equation:

$$\frac{1}{r} = \frac{1}{Kn} \cdot \frac{1}{C} + \frac{1}{n} \quad (4)$$

When $\frac{1}{r}$ is plotted against $1/C$, a straight line is obtained with a slope being $1/Kn$ and the ordinate intercept $1/n$.

Kn was calculated by using this equation, and free energy (ΔF) was calculated by the following equation:

$$\Delta F = -RT \ln Kn \quad (5)$$

where n is the number of binding sites on each protein molecules.

RESULTS AND DISCUSSION

In the case of erythrosine, ε_2 was obtained from Fig. 1. The value of ε_2 obtained from the ordinate intercept was about 42,000. Molar

Table I. Calculation of BSA-bound Erythrosine at pH 7.4

ε_1: 80,590 ε_2: 42,000

Total concn of dye $\times 10^{-6}$M/l	concn of albumin $\times 10^{-6}$M/l	ε_{app} at 525nm	α	Concn of free dye $\times 10^{-6}$10M/l c	Conocn of bound dye $\times 10^{-6}$10M/l	Moles dye bound per mole protein r	$1/r$	$1/c \times 10^{-6}$
3.0	1.5	54,000	0.311	9.329	2.067	1.378	0.726	1,071,944
3.0	2.0	52,667	0.276	8.292	2.171	1.085	0.921	1,205,938
3.0	2.5	51,333	0.242	7.256	2.274	0.910	1.099	1,378,214
3.0	3.0	50,000	0.207	6.219	2.378	0.793	1.262	1,607,917
5.0	1.5	56,000	0.363	1.814	3.186	2.124	0.471	551,286
5.0	2.0	54,200	0.316	1.581	3.419	1.710	0.585	632,623
5.0	2.5	52,800	0.280	1.399	3.601	1.440	0.684	714,630
5.0	3.0	51,600	0.249	1.244	3.756	1.252	0.799	803.958
7.0	1.5	57,714	0.407	2.851	4.150	2.766	0.362	350,818
7.0	2.0	55,000	0.337	2.358	4.642	2.321	0.431	424,066
7.0	2.5	53,143	0.289	2.021	4.979	1.992	0.502	494,744
7.0	3.0	52,143	0.263	1.840	5.160	1.720	0.581	543,521

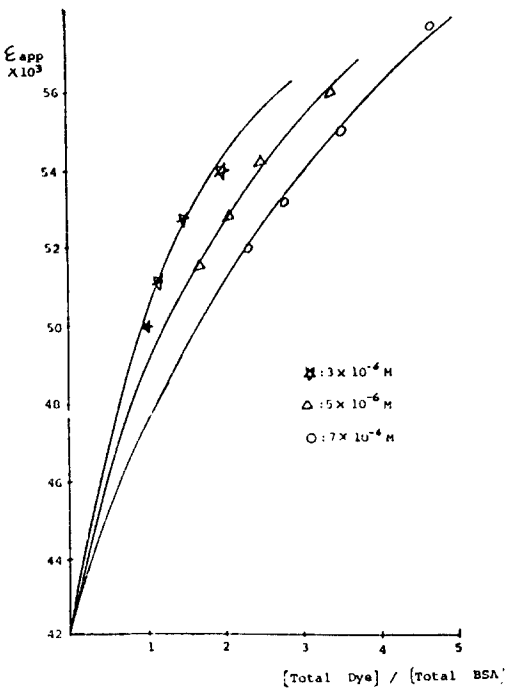

Fig. 1. Graphic determination of ε_2 for Erythrosine in the presence of bovine serum albumin at pH 7.4

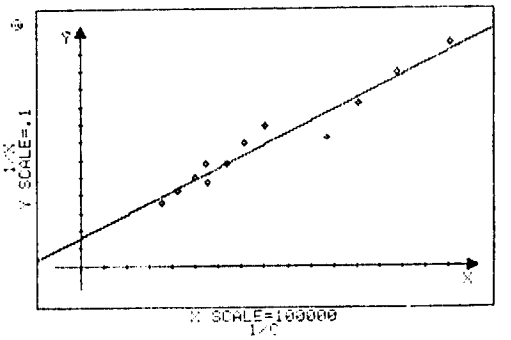

Fig. 2. Binding of Erythrosine by bovine serum albumin at pH 7.4

absorptivity of dye was about 80,590.

Data obtained from protein/dye ratio were expressed in Table I.

For the purpose of obtaining Kn, $1/r$ was plotted against $1/C$ in Fig. 2. The number of binding sites (n) was about 6.06, and binding free energy (ΔF) was about $-8,139$ cal/mole.

In the case of amaranth, ε_2 obtained from the ordinate intercept of Fig. 3 was 15,000 and molar absorptivity of dye was about 22,810.

Table II. Calculation of BSA-bound Amaranth at pH 7.4

ε_1: 22,810 ε_2: 15,000

Total concn Of dye $\times 10^{-5}$M/l	Concn of albumin $\times 10^{-5}$M/l	ε_{app} at 520nm	α	Concn of free dye $\times 10^{-5}$M/l c	Concn of bound dye $\times 10^{-5}$M/l	Moles dye bound per mole protein r	$1/r$	$1/c \times 10^{-5}$
3.0	1.5	19,800	0.615	1.844	1.156	0.771	1.297	54,236
3.0	2.0	18,967	0.508	1.524	1.476	0.738	1.355	65,630
3.0	2.5	18,300	0.423	1.268	1.732	0.693	1.443	78,889
3.0	3.0	17,867	0.367	1.101	1.899	0.633	1.580	90,814
5.0	1.5	20,540	0.709	3.547	1.453	0.967	1.032	28,195
5.0	2.0	19,960	0.635	3.175	1.825	0.912	1.096	31,492
5.0	2.5	19.300	0.551	2.753	2.247	0.899	1.113	36,326
5.0	3.0	18,800	0.487	2.433	2.567	0.856	1.169	41,105
7.0	1.5	21,214	0.796	5.570	1.430	0.954	1.049	17,954
7.0	2.0	20,657	0.724	5.070	1.930	0.965	1.037	19,722
7.0	2.5	20,143	0.658	4.610	2.391	0.956	1.046	21,694
7.0	3.0	19,657	0.596	4.174	2.826	0.942	1.062	23,957

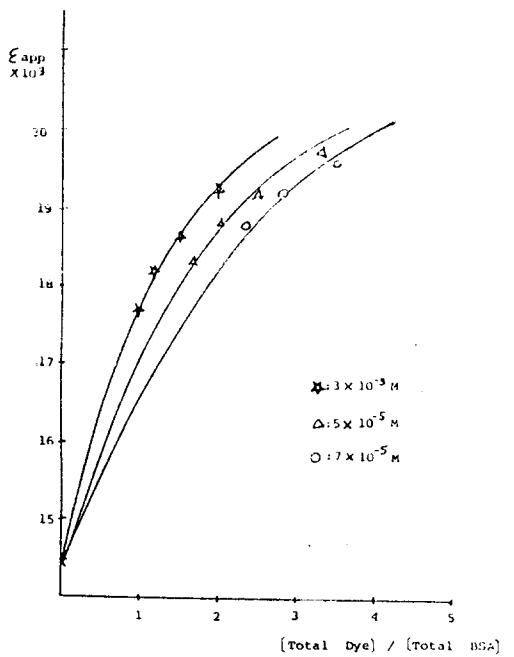

Fig. 3. Graphic determination of ε_2 for Amaranth in the presence of bovine sernm albumin at pH 7.4

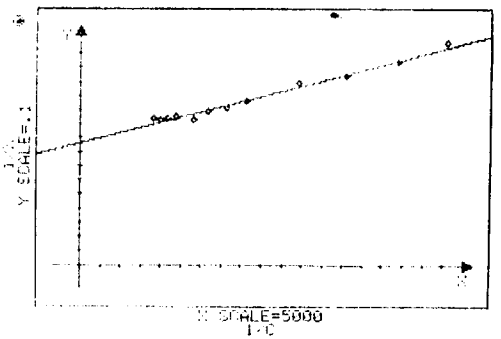

Fig. 4. Binding of Amaranth by bovine serum albumin at pH 7.4

By using these data, we calculated $1/r$ and $1/C$ values as shown in Table II and drew up Fig. 4.

The number of binding sites(n) and ΔF are about 1.16 and $-6,757$cal/mole, respectively. When comparing the binding intensities of edible dyes to protein in the range of concentration studied, erythrosine was stronger than amaranth at a constant pH.

In the case of tartrazine, ε_2 obtained from

Table III. Calculation of BSA-bound Tartrazine at pH 7.4

ε_1: 21,870 ε_2: 14,500

Total concn of dye $\times10^{-5}$M/l	Concn of albumin $\times10^{-5}$M/l	ε_{app} at 428nm	α	Concn of free dye $\times10^{-5}$M/l c	Concn of bound dye $\times10^{-5}$M/l	Moles dye bound per mole protein r	$1/r$	$1/c\times10^{-5}$
3.0	1.5	19,267	0.647	1.940	1.060	0.707	1.415	51,538
3.0	2.0	18,633	0.561	1.683	1.318	0.659	1.518	59,435
3.0	2.5	18,200	0.502	1.506	1.494	0.598	1.673	66,396
3.0	3.0	17,767	0.443	1.330	1.670	0.557	1.796	75,204
5.0	1.5	19,700	0.706	3,528	1.472	0.982	1.019	28,346
5.0	2.0	19,200	0.638	3,189	1.811	0.906	1,104	31,362
5.0	2.5	18,860	0.592	2.958	2.012	0.817	1.224	33,807
5.0	3.0	18,380	0.527	2.632	2.368	0.789	1.267	37,990
7.0	1.5	20,029	0.750	5.251	1.749	1.166	0.858	19,044
7.0	2.0	19,614	0.694	4.858	2.143	1.071	0.934	20,587
7.0	2.5	19,243	0.644	4.505	2.495	0.998	1.002	22,199
7.0	3.0	18,800	0.583	4.084	2.916	0.972	1.029	24,485

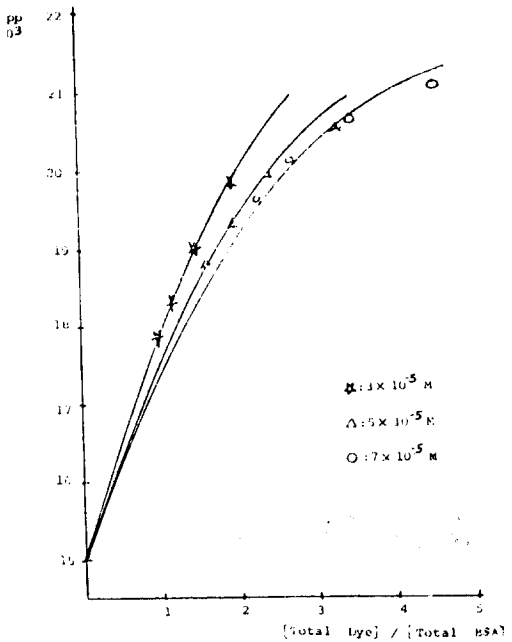

Fig. 5. Graphic determination of ε_2 for Tartrazine in the presence of bovine serum albumin at pH 7.4

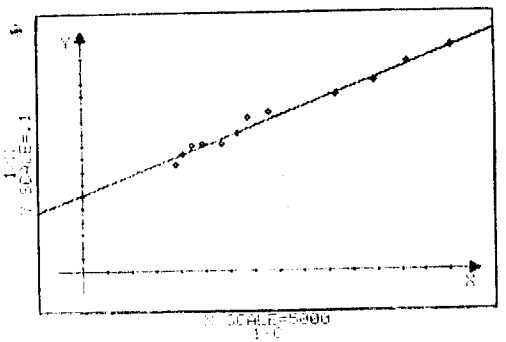

Fig. 6. Binding of Tartrazine by bovine serum albumin at pH 7.4

Fig. 5 was about 14,500 and molar absorptivity of dye was about 21,870.

We calculated 1/r and 1/c as in Table III and drew up Fig. 6 by using the same method.

The number of binding sites(n) was 1.61, and ΔF calculated by using Kn was about −6,333 cal/mole. When comparing the results obtained from the experiment, the binding intensity was increased in the order of tartrazine, amaranth and erythrosine.

Table IV. Calculation of BSA-bound sunset yellow at pH 7.4

ε_1: 20,300 ε_2: 13,000

otal concn of dye 10^{-6}M/1	Concn of albumin $\times 10^{-6}$M/1	ε_{app} at 480nm	α	Concn of free dye $\times 10^{-5}$M/1 c	Concn of bound dye $\times 10^{-5}$M/1	Moles dye bound per mole protein r	$1/r$	$1/c \times 10^{-5}$
3.0	1.5	17,533	0.621	1.863	1.137	0.758	1.319	53,676
3.0	2.0	16,900	0.594	1.603	1.397	0.699	1.431	62,393
3.0	2.5	16,400	0.466	1.397	1.603	0.641	1.560	71,569
3.0	3.0	16,033	0.416	1.247	1.753	0.585	1.711	80,220
5.0	1.5	17,600	0.630	3.151	1.849	1.233	0.811	31,739
5.0	2.0	16,880	0.532	2.658	2.343	1.171	0.854	37,629
5.0	2.5	16,520	0.482	2,411	2,589	1.036	0.966	41,477
5.0	3.0	16,140	0.430	2.151	2.849	0.950	1.053	46,497
7.0	1.5	18,057	0.693	4.849	2,151	1.434	0.697	20,621
7.0	2.0	17,500	0.614	4.315	2.685	1.343	0.745	23,175
7.0	2.5	17,100	0.562	3,932	3.069	1,227	0.815	25,436
7.0	3.0	16,686	0.505	3.534	3.466	1.155	0.866	28,295

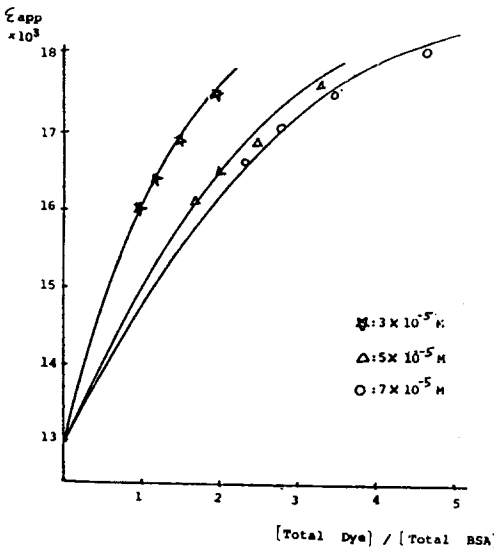

Fig. 7. Graphic determination of ε_2 for Sunset yellow in the presence of bovine serum albumin at pH 7.4

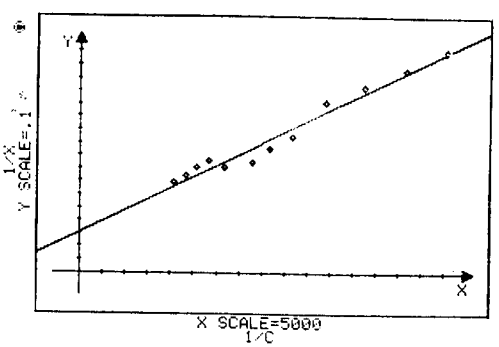

Fig. 8. Binding of Sunset yellow by bovine Serum albumin at pH 7.4

In the case of sunset yellow, ε_2 obtained from Fig. 7 was 13,000, and molar absorptivity of dye (ε_2) was about 20,300.

Data calculated by using these values and ε_{app} obtained from a vaious ratio of protein/dye are arranged in Table IV. 1/C in x-axis is plotted against 1/r in Y-axis as in Fig. 8.

The number of binding sites(n) was about 3.17 and the value of ΔF was about $-6,276$ cal/mole. This value is resemble to that of tartrazine.

SUMMARY

The binding free energy of edible dye bou to protein were measured and calculated means of spectrophotometry. Amaranth, eryt rosine, tartrazine and sunset yellow as edil dyes and bovine serum albumin as protein we used in this study.

The concentration ranges of edible dyes we $3\sim7\times10^{-6}$M in the case of erythrosine a: $3\sim7\times10^{-5}$M in the case of amaranth, tartrazi and sunset yellow. The binding free energy edible dye bound to BSA is ranged $-6,300$ $-8,100$cal/mole.

The edible dyes arranged in the decreasi order of binding intensities followe dthe orde in which the maximum wavelength of edil dye decreased:

Erythrosine>Amaranth>Tartrazine≒Sunse Yellow

But quantitative relationship between tl maximum wavelength and binding intensi was not obvious at this stage.

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