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Studies on the Interaction of Edible Dyes with Protein (II). The effects of drug additions on protein binding of edible dyes

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Abstract □ The effect of drug addition on the bovine serum albumin (BSA)-edible dye complex was studied by spectrophotometric method. The edible dyes tested were amaranth, erythrosine, tatrazine and sunset yellow. The moles of bound dye per protein mole and free energies for edible dyes bounded were determined at pH 7.4. The values of free energy change by the addition of drugs to BSA-edible dye were ranged from -6,260 to -8,030 cal/mole. In the wide range of edible dye concentration ($0.3\text{-}7\times10^{-5}$ M), acetylsalicylic acid(ASA) showed pattern of displacement different from that of dye. It was assumed that ASA has different binding mechanisms from edible dye.

Keywords □ Bovine serum albumin, spectrophotometry, amaranth, erythrosine, tartrazine, sunset yellow, binding energy, binding site, drug addition.

The effect of drug binding to protein has been studied as a part of important field when we estimate the therapeutic effect of drug in the body. The phenomena of drug-protein binding and of competitive binding of drugs for available protein sites have been the subjects of many investigations and have been reviewed[1]. Various experimental procedures and analysis methods have been used to study drug-protein interactions. These include equilibrium dialysis[2], ultrafiltration[3], gel filtration[4], NMR rate measurements[5], fluorescence techniques[6] and spectrophotometry[7,8].

There is often enhanced the change of absorption spectrum in the presence of protein. This change come from the interaction of both molecules. In this case, absorption maximum is shifted to longer or shorter wavelength side, and absorbance is decreased or increased. Based on these phenomena, the authors previously investigated the binding parameters to the formation of intermolecular complex[9].

In this paper, the authors present the changes of the binding parameters of BSA-edible dye in the presence of drugs using spectrophotometric method.

EXPERIMENTAL METHODS

Materials and Apparatus

BSA (m.w. 68,000) was obtained from Sigma Chemical Co. St. Louis, Mo. Amaranth (Red No. 2), erythrosine (Red No.3), tatrazine (yellow No. 4) and sunset yellow (yellow No.5) were recrystallized several times from water or ethanol.

All other materials were of reagent grade, and solutions were prepared in deionized and distilled water. All solutions were prepared in 0.2 M-phosphate buffer of pH 7.4 at 22℃. Absorption measurements were carried out with LKB-Apple II using 10-mm cells.

Procedure

We have previously reported that spectrophotometric method is a useful technique to determine binding parameters between edible dye and protein[9].

Experimental method was carried out on the basis of previous report. The final concentrations of erythrosine used in this experiment were $3\text{-}7\times10^{-6}$ M, and those of BSA were prepared to $1.5\text{-}3\times10^{-6}$ M.

In the case of amaranth, tatrazine, and sunset yellow, the final concentrations of these dyes were $3\text{-}7\times10^{-5}$ M, and those of BSA were prepared to $1.5\text{-}3.0\times10^{-5}$ M. The concentration of drug was 5.0×10^{-3} M.

RESULTS

Based on this binding parameters between edible dye and protein, we investigated the effect of drug addition on edible dye-protein binding.

Table I summarizies the absorbance changes of edible dyes measured when drugs are added or not in the presence of BSA. As shown in the above table,

Table I. Absorbance changes obtained by drug addition to BSA-dye binding.

	Erythrosine	Amaranth	Tatrazine	S.Y.
dye	0.227	0.700	0.658	0.597
dye-BSA	0.137	0.590	0.585	0.499
dye-BSA-urea	0.141	0.605	0.590	0.509
dye-BSA-chloramphenicol	0.132	0.600	0.603	0.518
dye-BSA-meprobamate	0.133	0.594	0.581	0.503
dye-BSA-sulpyrin	0.140	0.585	0.583	0.492
dye-BSA-acetaminophen	0.137	0.600	0.581	0.522
dye-BSA-acetanilide	0.139	0.592	0.602	0.491
dye-BSA-ASA	0.174	0.657	0.642	0.532
dye-BSA-diethylbarbital	0.137	0.593	0.587	0.509

Table II. Effects of ASA addition to edible dye.

	Erythrosine	Amaranth	Tatrazine	Sunset Y.
λ_{max} (nm)	529	520	428	480
dye	0.246	0.700	0.629	0.669
dye-ASA	0.249	0.703	0.630	0.671

when drugs are added to dye-protein solution, for example, urea, diethylbarbital, chloramphenicol, meprobamate, sulpyrin, acetaminophen, acetanilide and ASA, it can be assumed that there is any interaction between them because of the apparent absorbance difference.

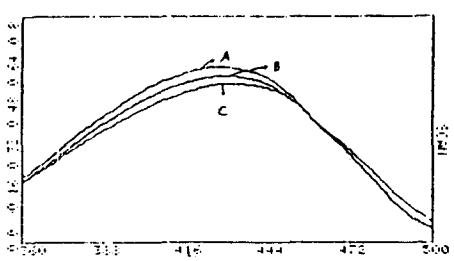

Fig.1. Visible spectrum of tatrazine.
A: dye, B: dye+BSA+ASA, C: dye+BSA, dye: 3×10^{-5} M, ASA: 5×10^{-5} M, BSA: 3×10^{-5} M

Table III. The changes of absorbance by concentration dependence of ASA to BSA-dye.

	Erythrosine	Amaranth	Tatrazine	Sunset Y.
dye	0.246	0.700	0.629	0.669
dye-BSA	0.147	0.581	0.529	0.597
dye-BSA-5×10^{-3}M ASA	0.204	0.666	0.570	0.643
dye-BSA-ASA sat. soln.	0.200	0.669	0.571	0.646

Dye: 3×10^{-5} (in the case of Erythrosine, 3×10^{-6} M)
BSA: 3×10^{-5} M(in the case of Erythrosine, 3×10^{-6} M)

In order to clarify the effect of drug addition between them, the authors investigated furthermore using ASA as drug since it is expected to give rise to a marked absorption change than the other

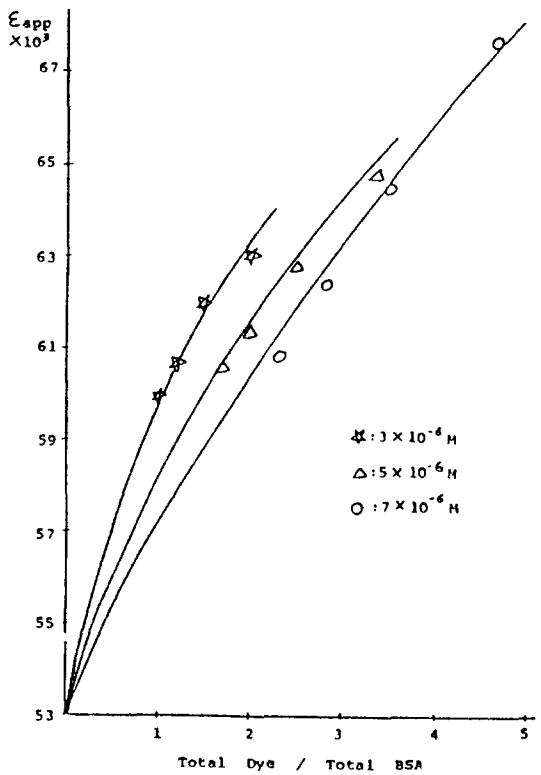

Fig.2. Graphic determination of ε_2 for BSA-erythrosine in the presence of ASA at pH 7.4.

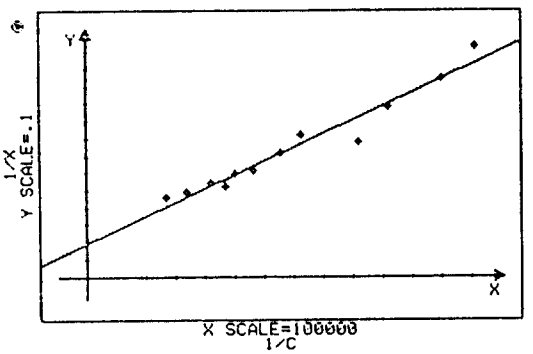

Fig.3. Binding of erythrosine by bovine serum albumin in the presence of ASA at pH 7.4

drugs.

We measured the absorbance changes of edible dye at λ_{max} in order to check the effect of ASA addition to edible dye.

As shown in Table II, we can see that there is almost not any interaction between ASA and edible dye. We also checked the absorbance changes by the addition-order and the elapsed-time of edible dye or ASA bound to BSA, but there are no changes due to the interaction between them.

On this solution of edible dye-BSA, ASA was added to be 5×10^{-3} M(final concentration) by using 0.2 M-phosphate buffer of pH 7.4.

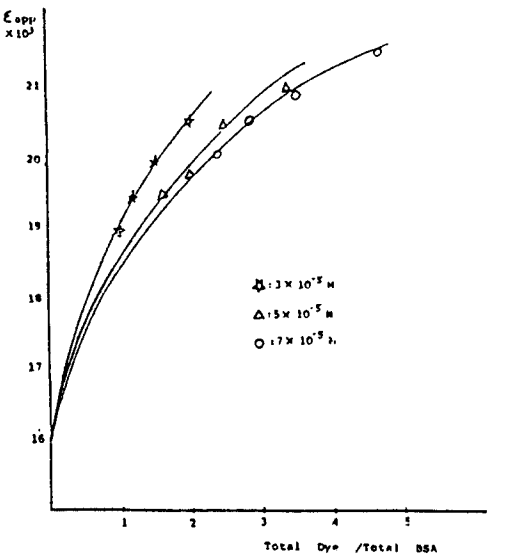

Fig.4. Graphic determination of ε_2 for BSA -amaranth in the presence of ASA at pH 7.4.

Fig.1 shows the absorbance difference of dye -BSA solution in the absence and presence of ASA. Curve C is the absorption spectrum of edible dye decreased at λ_{max} by the combination of edible dye and BSA, Curve B is the absorption spectrum of edible dye increased at λ_{max} by the addition of

Table IV. Calculation of BSA-bound erythrosine in the presence of ASA at pH 7.4.

Total Concn of Dye $\times10^{-6}$M/l	Concn of albumin $\times10^{-6}$M/l	ε_{app} at 525nm	α	Concn of free Dye $\times10^{-6}$M/l c	Concn of bound Dye $\times10^{-6}$M/l	Moles Dye bound per mole protein r	1/r	$1/c\times10^{-6}$
3.0	1.5	63,000	0.363	1.087	1.913	1.275	0.784	919,667
3.0	2.0	62,000	0.326	9.786	2.021	1.011	0.989	1,021,852
3.0	2.5	60,667	0.278	8.336	2.166	0.867	1.154	1,199,565
3.0	3.0	60,000	0.254	7.612	2.239	0.746	1.340	1,313,810
5.0	1.5	64,800	0.428	2.139	2.862	1.908	0.524	467,627
5.0	2.0	62,800	0.355	1.776	3.224	1.612	0.620	563,061
5.0	2.5	61,400	0.305	1.522	3.478	1.391	0.719	656,905
5.0	3.0	60,600	0.276	1.377	3.623	1.208	0.828	726,053
7.0	1.5	67,714	0.533	3.733	3.267	2.178	0.459	267,864
7.0	2.0	64,571	0.419	2.936	4.064	2.032	0.492	340,614
7.0	2.5	62,429	0.342	2.392	4.608	1.843	0.543	418,030
7.0	3.0	60,857	0.285	1.994	5.007	1.669	0.599	501,636

ε_1 : 80,590, ε_2 : 53,000

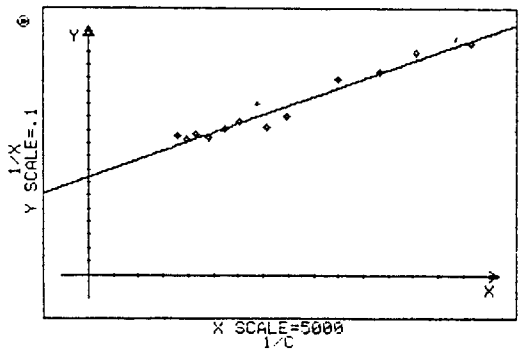

Fig.5. Binding of amaranth by bovine serum albumin in the presence of ASA at pH 7.4

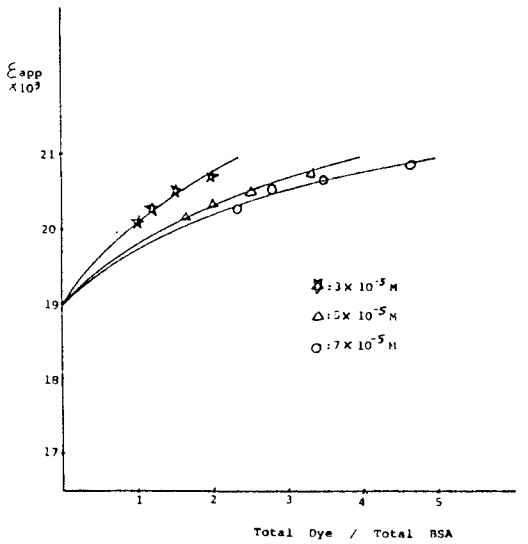

Fig.6. Graphic determination of ε_2 for BSA -bound tartrazine in the presence of ASA at pH 7.4.

ASA.

In order to investigate, furthermore, the phenomenon of increased absorbance by the addition of ASA, we measured the absorbance changes when 5×10^{-3} M-ASA solution and the saturated solution are added to a constant concentration of BSA-dye solution, respectively. These results are shown in Table III.

Table III shows the absorbances of dissociated dyes enhanced by the interaction between a constant BSA-dye solution and several concentrations of ASA. These are not exceed the absorbance of edible dye itself. Thus, it may be considered that ASA and dye competitively bind to the same

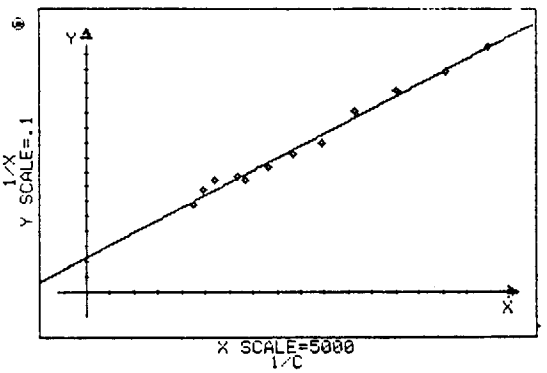

Fig.7. Binding of tartrazine by bovine serum albumin in the presence of ASA at pH 7.4

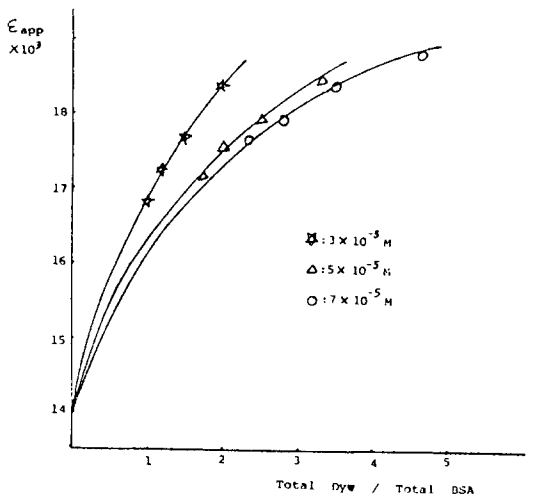

Fig.8. Graphic determination of ε_2 for BSA -sunset yellow in the presence of ASA at pH 7.4.

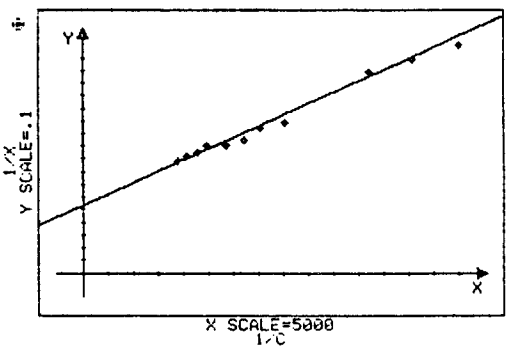

Fig.9. Binding of sunset yellow by bovine serum albumin in the presence of ASA at pH 7.4

Table V. Calculation of BSA-bound amaranth in the presence of ASA at pH 7.4.

Total Concn of Dye $\times 10^{-5}$M/l	Concn of albumin $\times 10^{-5}$M/l	ε_{app} at 520 nm	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	20,533	0.666	1.997	1.003	0.669	1.496	50,074
3.0	2.0	19,900	0.573	1.718	1.282	0.641	1.560	58,205
3.0	2.5	19,467	0.509	1.527	1.473	0.589	1.698	65,481
3.0	3.0	18,967	0.436	1.307	1.693	0.564	1.772	76,517
5.0	1.5	20,980	0.731	3.656	1.344	0.896	1.116	27,349
5.0	2.0	20,480	0.658	3.289	1.711	0.855	1.169	30,402
5.0	2.5	19,800	0.558	2.790	2.210	0.884	1.131	35,842
5.0	3.0	19,440	0.505	2.526	2.474	0.825	1.212	39,593
7.0	1.5	21,443	0.799	5.595	1.405	0.937	1.067	17,874
7.0	2.0	20,929	0.724	5.066	1.934	0.967	1.034	19,739
7.0	2.5	20,557	0.669	4.684	2.316	0.926	1.080	21,348
7.0	3.0	20,043	0.594	4.156	2.844	0.948	1.055	24,064

ε_1 : 22,810, ε_2 : 16,000

Table VI. Calculation of BSA-bound tartrazine in the presence of ASA at pH 7.4.

Total Concn of Dye $\times 10^{-5}$M/l	Concn of albumin $\times 10^{-5}$M/l	ε_{app} at 428nm	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	20,700	0.592	1.777	1.223	0.815	1.227	56,275
3.0	2.0	20,467	0.511	1.533	1.467	0.733	1.364	65,227
3.0	2.5	20,267	0.441	1.324	1.676	0.670	1.492	75,526
3.0	3.0	20,133	0.395	1.185	1.815	0.605	1.653	84,412
5.0	1.5	20,720	0.599	2.997	2.004	1.336	0.749	33,372
5.0	2.0	20,500	0.523	2.613	2.387	1.193	0.838	38,267
5.0	2.5	20,320	0.460	2.300	2.700	1.080	0.926	43,485
5.0	3.0	20,160	0.404	2.021	2.979	0.993	1.007	49,483
7.0	1.5	20,829	0.637	4.460	2.540	1.693	0.591	22,422
7.0	2.0	20,686	0.587	4.112	2.889	1.444	0.692	24,322
7.0	2.5	20,514	0.528	3.693	3.307	1.323	0.756	27,075
7.0	3.0	20,286	0.448	3.136	3.864	1.288	0.776	31,889

ε_1 : 21,870 ε_2 : 19,000

:ationic binding sites[10] or the other binding sites)n BSA.

The measurement of ε_2 for the binding of ASA o BSA-dye are shown in Fig.2. ε_2 obtained from this graph is about 53,000, on the basis of this value, the parameters were calculated by using ε_{app} obtained at various ratio of BSA and dye are summerized in Table IV.

Table VII. Calculation of BSA-bound sunset yellow in the presence of ASA at pH 7.4.

Total Concn of Dye $\times 10^{-5}$M/l	Concn of albumin $\times 10^{-5}$M/l	ε_{app} at 428nm	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	18,367	0.693	2.079	9.206	0.614	1.629	48,092
3.0	2.0	17,667	0.582	1.746	1.254	0.627	1.595	57,273
3.0	2.5	17,200	0.508	1.524	1.476	0.591	1.694	65,625
3.0	3.0	16,800	0.444	1.333	1.667	0.556	1.800	75,000
5.0	1.5	18,420	0.702	3.508	1.492	0.995	1.005	28,507
5.0	2.0	17,900	0.619	3.095	1.905	0.952	1.050	32,308
5.0	2.5	17,560	0.565	2.825	2.175	0.870	1.150	35,393
5.0	3.0	17,120	0.495	2.476	2.524	0.841	1.189	40,385
7.0	1.5	18,786	0.760	5.318	1.683	1.122	0.892	18,806
7.0	2.0	18,357	0.692	4.841	2.159	1.079	0.926	20,656
7.0	2.5	17,929	0.624	4.365	2.635	1.054	0.949	22,909
7.0	3.0	17,629	0.576	4.032	2.968	0.989	1.011	24,803

ε_1 : 20,300 ε_2 : 14,000

Table VIII. Difference of ΔF and n in the abscence of and in the presence of ASA.

	in the abscence of ASA		in the presence of ASA	
	ΔF (K cal/mole)	n	ΔF	n
BSA-Erythrosine	-8,139	6.06	-8,035	5.12
BSA-Amaranth	-6,757	1.15	-5,654	1.33
BSA-Tatrazine	-6,333	1.61	-6,296	4.22
BSA-Sunset Y.	-6,276	3.17	-6,265	1.82

As in Fig.3, 1/C in X-axis is plotted against 1/r in Y-axis. The line is a good linear in the range of concentration studied. The number of binding sites is about 5, 1?, and ΔF calculated using these parameters is about -8,035 cal/mole. The results obtained by using the same experimental method in the case of Amaranth, Tatrazine and Sunset yellow are summerized in Figs.4-9 and Tables V-VII.

We calculated the free binding energies (ΔF) and number of binding sites (n) using the above data, and compared the values obtained in the absence and presence of ASA.

And shown in Table VIII, the values of binding free energies to erythrosine, amaranth, tatrazine and sunsetyellow by the addition of ASA are decreased as about 100, 1,100, 40 and 10 cal/mole, respectively.

DISCUSSION

Based on the binding parameter obtained for dye-BSA complex, we calculated the binding free energy of BSA-dye when ASA are added to its solution. The edible dyes arranged in the decreasing magnitude of binding free energy is as follows:

Amaranth>erythrosine>tartrazine>sunset yellow

Binding free energies of ASA or edible dye to BSA were almost similar[11].

And there is a possibility that these materials bind to the same sites or the other sites of BSA. But further study is required to clarify the mechanisms of protein binding of drugs and dyes on the basis of their competitive interactions. The results of this study may be helpful to clarify the fact that increased blood levels of free dyes due to competitive or selective binding with drugs are responsible for hyperkinetic disorders of childeren who take a large amount of colored food and beverages[12,13,14].

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