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BINDING OF 1,2,3,4,6-PENTAGALLOYLGLUCOSE TO PROTEINS, LIPIDS, NUCLEIC ACIDS AND SUGARS

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Key Word Index—Tannin; binding; biological substances; cultured cells; Sephadex G-100 column; inhibition of biological activity.

Abstract—The binding of 1,2,3,4,6-pentagalloylglucose (PGG) to various proteins, lipids, nucleic acids, sugars and cultured human amniotic cells (FL cells) was examined quantitatively. The results indicate that PGG binds less to acidic than to neutral or basic proteins, but more to basic phospholipids than the other lipids, and also to nucleic acids and sugars. On the other hand, PGG bound to FL cells irreversibly and the antiherpetic or cytotoxic activity of PGG was inhibited in the presence of L-α-phosphatidylcholine or bovine serum albumin.

INTRODUCTION

We have previously reported that pentagalloylglucose (PGG) has antiherpetic activity [1–3]. In this work, we have tried to determine quantitatively its binding to various biological substances. Much work on the binding of tannin to proteins [4, 5], alkaloids [6] and metals [7] has been reported. Measurement of proteins [4, 5] and tannins [8] in the supernatants was carried out in order to determine the tannin–protein binding affinity, and specificity of the proanthocyanidin–protein interaction was investigated using a competitive binding assay [9]. But since these studies were incomplete, we tried to examine quantitatively the binding of PGG by determining the amount of free PGG in the supernatant after complex formation.

RESULTS

A representative Sephadex G-100 column chromatographic pattern of the supernatant of a PGG–biological substance mixture gives in the first peak the free substance, a second peak due to the soluble complex and a third peak for free PGG (retention time 45 min). The PBD_{50} values (see Experimental) of various test substances are listed in Table 1.

10^6 FL cells bound about 20 μg of PGG for 20 hr and no PGG was released from PGG-bound FL cells. In order to elucidate the relationship between the binding affinity and the inhibition of the biological activity of PGG, we examined the effect of high (BSA, PC) and low (pepsin, cholesterol) binding affinity substances on the antiherpetic or cytotoxic activity of PGG. The PID_{50} (see Experimental) and SRD_{50} (see Experimental) values of these substances are listed in Table 2.

DISCUSSION

When pentagalloylglucose was mixed with a basic protein, for example, lysozyme or histone, there was little precipitate, but the PBD_{50} values were as low as that for bovine serum albumin which formed a large precipitate with PGG. Thus the amount of precipitate is related to the solubility of the complex and not to the binding affinity to PGG. Therefore, the tannin binding affinity cannot be determined by the amount of precipitate or by the amount of proteins [4, 5] or tannins [8] in the supernatants when soluble complexes are present in the supernatants. Furthermore, the specificity of the proanthocyanidin–protein interaction should not be investigated using a competitive binding assay [9] because the specificity is not as marked as that in antigen–antibody interactions. Also, in the case of metals or alkaloids, we intend to re-examine the tannin binding affinity.

Pentagalloylglucose attaches itself to particles of Biogel, Sephadex (except for G-100) or Toyo Pearl, but we succeeded in determining the amount of free PGG in the eluant from a Sephadex G-100 column. As shown in Table 1, acidic proteins (isoelectric point < 4) bound to PGG less than neutral or basic proteins, and basic phospholipids bound to PGG more than to the other lipids. Therefore, ionic bonds appear to participate in the PGG binding, but since the more condensed polylysine had the higher binding affinity and BSA, which had no charge at pH 5, bound to PGG more than basic proteins, such as cytochrome *c*, hydrogen bonds must also take part in the binding of PGG [10]. Since the homopolymer of lysine or glucose is bound more to PGG than the monomer, the more condensed homopolymer will bind the greater amount of PGG.

Because one FL cell bound 2×10^{-11} g (2×10^{-14} mol) of PGG, it can be deduced that one FL cell binds about 10^{10} molecules of PGG under these experimental conditions, and therefore PGG molecules may be superimposed on a FL cell surface. It is possible that the basic phospholipids on the outer side of the cell membrane are binding sites to PGG, besides the cell membrane proteins. Because no PGG was released from PGG-bound FL cells, the PGG binding to FL cells is irreversible. As shown in Table 2, the substances which have the higher binding affinity to PGG also inhibited to a greater extent the antiherpetic or cytotoxic activity of PGG.

Table 1. PBD_{50} values of various test substances against pentagalloylglucose

Substances	M_r (k)	PBD_{50} (μg/ml)	Substances	M_r (k)	PBD_{50} (μg/ml)
Proteins			*Lipids*		
Pepsin	34	800	L-α-Lecithin (PC)	0.83	30
Orosomucoid	44	> 1000 (39%)*	L-α-Lysolecithin	0.53	50
BSA	69	70	L-α-Cephalin	0.79	69
γ-Globulin	170	110	Sphingomyelin	0.75	67
Haemoglobin	65	40	L-α-Phosphatidylinositol	0.91	> 1000 (30%)*
Myoglobin	17	70	L-α-Phosphatidylserine	0.83	> 1000 (33%)*
α-Chymotrypsin	25	180	Cholesterol	0.39	> 1000 (10%)*
α-Chymotrypsinogen A	26	140	Ganglioside	1.7	> 1000 (18%)*
Cytochrome *c*	12	270			
Trypsin	23	80	Nucleic acids		
Histone	15	80	ATP	0.51	> 1000 (1%)*
Lysozyme	14	70	Thymidine	0.24	> 1000 (33%)*
			DNA	10000	350
Amino acids and polymers			RNA	1000	340
L-Arginine	0.18	> 1000 (10%)*			
L-Lysine	0.15	> 1000 (13%)*	Sugars		
Poly-L-lysine	4	> 1000 (47%)*	Glucose	0.18	> 1000 (8%)*
Poly-L-lysine	14	330	Maltose	0.34	> 1000 (10%)*
Poly-L-lysine	21	350	Maltotriose	0.50	> 1000 (20%)*
Poly-L-lysine	55	45	Maltotetraose	0.67	> 1000 (24%)*
Poly-L-lysine	200	10	Maltopentaose	0.83	> 1000 (29%)*
L-Threonine	0.12	> 1000 (12%)*	Maltohexaose	0.99	> 1000 (34%)*
Glycine	0.08	> 1000 (9%)*	Maltoheptaose	1.2	1000
L-Serine	0.11	> 1000 (7%)*	Glycogen	300	910
L-Glutamic acid	0.14	> 1000 (7%)*	Cellulose	400	> 1000 (22%)*
L-Aspartic acid	0.13	> 1000 (3%)*	Starch	100	260
Poly-L-aspartic acid	36	> 1000 (10%)*			

* Percentage of bound PGG at 1000 μg/ml of test substance.

Table 2. PID_{50} and SRD_{50} values of BSA, PC, pepsin and cholesterol

	BSA	PC	Pepsin	Cholesterol
PID_{50} (μg/ml)	2.0	0.7	> 10	> 10
SRD_{50} (μg/ml)	2.5	1.0	10	> 10

EXPERIMENTAL

Pentagalloylglucose was isolated from the galls of *Rhus javanica* according to the method described previously [2]. The other substances were obtained from Sigma. All other chemicals were commercial preparations of the highest quality available.

Binding assay to biological substances. 0.2 ml of PGG soln (0.2 mg/ml 0.1 M acetate buffer, pH 5 = buffer A) was mixed with each 0.2 ml of buffer A containing various amounts of the test substance and shaken at 30° for 20 hr. Then the incubation mixtures were centrifuged at 2000 *g* for 15 min. Each 0.1 ml of the supernatants was applied to a Sephadex G-100 column (0.5 × 7 cm) equilibrated with buffer A. The elution was carried out with buffer A at a flow rate of 0.22 ml/min for 90 min at room temp. The eluate was monitored at 280 nm using a UV monitor II manufactured by ATTO Co., Japan. The amount of free PGG in the supernatant was derived from the peak area and that of bound PGG from subtracting that of free PGG from that of original PGG. The binding affinity was expressed as 50% PGG binding dose (PBD_{50}), i.e. the concn of biological substance required to bind to 50% of the amount of original PGG.

Binding assay to FL cells. FL cells were grown in Eagle MEM medium containing 5% calf serum as monolayers at 36°. The cells were dispersed with 0.2% trypsin and suspended in 0.15 M acetate buffer, pH 5 (= buffer B). 0.2 ml of PGG soln (0.2 mg/ml buffer B) was added to 0.2 ml of FL cells suspension (10^6 cells). After shaking for 20 hr at 30°, the suspension was centrifuged at 500 *g* for 5 min. The amount of bound PGG was estimated as noted above. To examine the reversibility, the pellet was re-suspended in buffer B, shaken for 3 hr at 30°, and then centrifuged. The amount of free PGG in the supernatant was detected using a Sephadex G-100 column.

Effect of BSA, PC, pepsin and cholesterol on the antiherpetic or cytotoxic activity of PGG. The antiherpetic or cytotoxic assay was carried out according to the method described previously [3]. The inhibitory effect of compound on the antiherpetic activity of PGG was expressed as PID_{50}, i.e. the concn of compound required to increase the number of plaques, in the presence of 20 μg/ml PGG, to 50% of that in the control cell cultures. The inhibitory effect of compound on the cytotoxic activity of PGG was expressed as SRD_{50}, i.e. the concn of compound required to reduce the cell staining proportion to 50% of that in the presence of 150 μg/ml PGG.

REFERENCES

1. Takechi, M. and Tanaka, Y. (1981) *Planta Med.* **42**, 69.
2. Takechi, M. and Tanaka, Y. (1982) *Planta Med.* **45**, 252.

3. Takechi, M., Tanaka, Y., Takehara, M., Nonaka, G. and Nishioka, I. (1985) *Phytochemistry* **24**, 2245.
4. Bate-Smith, E. C. (1973) *Phytochemistry* **12**, 907.
5. Okuda, T., Mouri, K. and Hayashi, N. (1976) *Yakugaku Zasshi* **96**, 1143.
6. Okuda, T., Mouri, K., Shioda, M. and Ida, K. (1982) *Yakugaku Zasshi* **102**, 735.
7. Okuda, T., Mouri, K. and Shioda, M. (1982) *Yakugaku Zasshi* **102**, 854.
8. Konishi, E. and Hotta, S. (1980) *Microbiol. Immunol.* **24**, 847.
9. Hagerman, A. E. and Butler, L. G. (1981) *J. Biol. Chem.* **256**, 4494.
10. Haslam, E. (1974) *Biochem. J.* **139**, 285.