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APPENDICES

APPENDIX A: Preparation for buffer solution

50 mM Sodium acetate buffer pH 3.0, 4.0, 4.5, 5.0, 5.5 and 6.0

CH_3COONa 0.4102 g

Adjusted pH to 4.0, 4.5, 5.0, 5.5 or 6.0 with glacial acetic acid and adjusted volume to 100 ml with distilled water.

50 mM Potassium phosphate buffer pH 6.0

KH_2PO_4 0.640 g

K_2HPO_4 0.0517 g

Adjusted volume to 100 ml with distilled water.

50 mM Potassium phosphate buffer pH 6.5

KH_2PO_4 0.567 g

K_2HPO_4 0.145 g

Adjusted volume to 100 ml with distilled water.

50 mM Potassium phosphate buffer pH 7.0

KH_2PO_4 0.417 g

K_2HPO_4 0.338 g

Adjusted volume to 100 ml with distilled water.

50 mM Potassium phosphate buffer pH 7.5

KH_2PO_4 0.227 g

K_2HPO_4 0.580 g

Adjusted volume to 100 ml with distilled water.

50 mM Tris-HCl pH 8.0 and 9.0

Tris (hydroxymethyl)-aminomethane 0.605 g

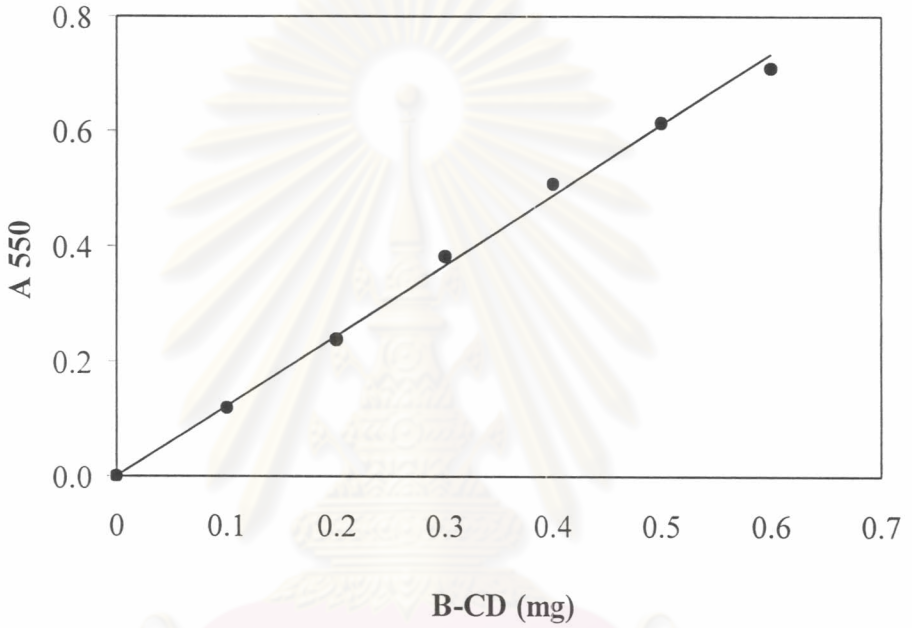
Adjusted to pH 8.0 or 9.0 with 1 M HCl and adjusted volume to 100 ml with distilled water.

50 mM Glycine-NaOH pH 10.0 and 11.0

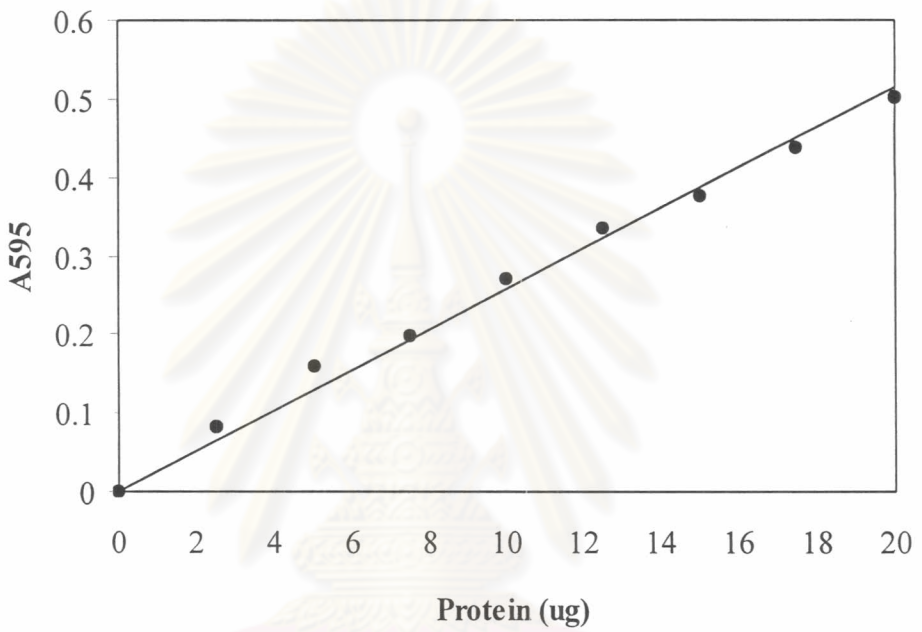
Glycine 0.375 g

Adjusted to pH 10.0 or 11.0 with 1 M NaOH and adjusted volume to 100 ml with distilled water.

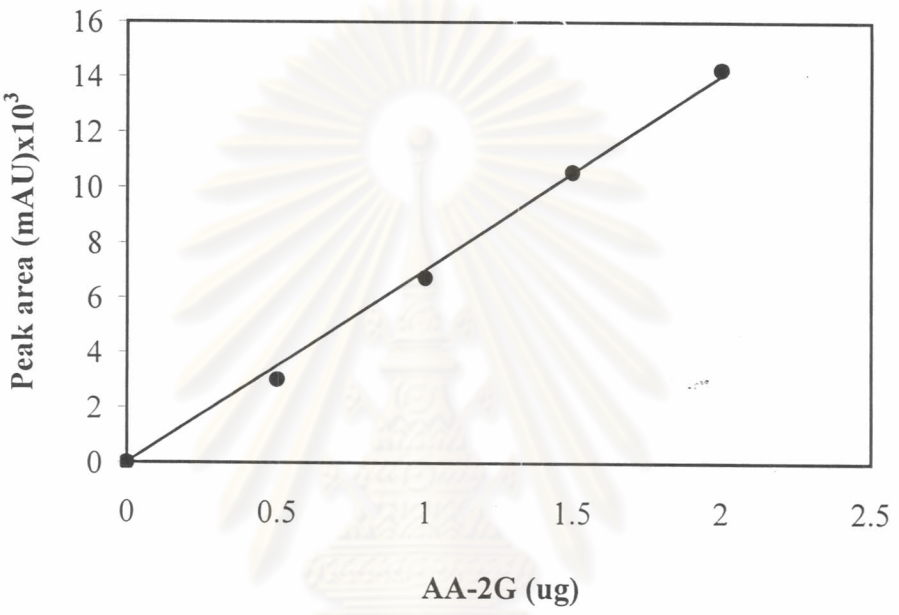
APPENDIX B: Standard curve of β-cyclodextrin by phenolphthalein method



APPENDIX C: Standard curve for protein determination by Bradford's method



APPENDIX D: Standard curve of ascorbic acid-2-glucoside by HPLC



APPENDIX E: Raw data

Suitable conditions for AA-2G production

Table E1 Effect of pH on AA-2G production

(4% β-CD, 4% AANa, 0.2% thiourea, pH 5.0-6.0, 40°C, 24 h in the dark)

pH	AA-2G formed (g/l)			Yield (%)
	1	2	Average	
5.0	0.740	0.746	0.746 ± 0.012	1.86
5.5	0.440	0.460	0.460 ± 0.026	1.15
6.0	0.445	0.416	0.416 ± 0.041	1.04

Table E2 Effect of temperature on AA-2G production

(4% β-CD, 4% AANa, 0.2% thiourea, pH 6.0, 30 - 40°C, 24 h in the dark)

Temperature (°C)	AA-2G formed (g/l)			Yield (%)
	1	2	Average	
30	0.544	0.364	0.364 ± 0.127	0.91
40	0.432	0.432	0.432 ± 0.021	1.08
50	0.435	0.211	0.323 ± 0.159	0.53

Table E3 Time-course of AA-2G production by immobilized CGTase

(4% β-CD, 4% AANa, 0.2% thiourea, pH 6.0, 40°C, 0-24 h in the dark)

Time (h)	AA-2G formed (g/l)				Yield (%)
	1	2	3	Average	
0	0	0	0	0	0
2	0.1175	0.1371	0.1561	0.137 ± 0.019	0.34
6	0.2164	0.2541	0.2412	0.237 ± 0.019	0.59
12	0.2721	0.3257	0.2221	0.273 ± 0.052	0.75
18	0.3185	0.3669	0.2927	0.326 ± 0.038	0.82
24	0.3494	0.4169	0.3494	0.372 ± 0.039	0.93
36	0.4726	0.4957	0.4303	0.466 ± 0.033	1.17
48	0.5442	0.5571	0.5061	0.536 ± 0.027	1.34

Table E4 Time course of AA-2G production by soluble CGTase

(4% β-CD, 4% AANa, 0.2% thiourea, pH 6.0, 40°C, 0-24 h in the dark)

Time (h)	AA-2G formed (g/l)			Yield (%)
	1	2	Average	
0	0	0	0	0
2	0.054	0.051	0.053 ± 0.002	0.13
6	0.140	0.108	0.124 ± 0.023	0.31
12	0.262	0.296	0.279 ± 0.024	0.70
18	0.466	0.532	0.499 ± 0.047	1.25
24	0.686	0.618	0.652 ± 0.048	1.63
36	1.074	0.807	0.940 ± 0.188	2.35
48	1.624	1.524	1.574 ± 0.070	3.93

Table E5 Effect of ascorbic acid concentration

(4% β-CD, 0.5 - 4% AANa, 0.2% thiourea, pH 6.0, 40°C, 24 h in the dark)

Ascorbic acid (%)	AA-2G formed (g/l)	Yield (%)
0.5	0.141	2.82
1	0.210	2.10
2	0.304	1.52
4	0.432	1.08
8	0.850	1.06
10	1.245	1.25

Table E6 Overall optimum condition

(4% β-CD, 1-4% AANa, 0.2% thiourea, pH 5.0, 40°C, 24 h in the dark)

Ascorbic acid (%)	AA-2G formed (g/l)	Yield (%)
1	0.259	2.59
2	0.584	2.92
4	0.726	1.81

Table E7 Batch reusability of immobilized CGTase on alumina for AA-2G production

(4% β-CD, 2% AANa, 0.2% thiourea, pH 5.0, 40°C, 24 h in the dark)

No. Utilization	AA-2G formed (g/l)				Residual activity (%)
	Untreated with AMG		Treated with AMG		
	AVG	SD	AVG	SD	
1	0.570	0.062	0.811	0.003	100.0
2	0.435	0.102	0.675	0.186	91.9
3	0.283	0.016	0.557	0.184	74.4

APPENDIX F: Washing of the immobilized enzyme

a) Reaction mixture

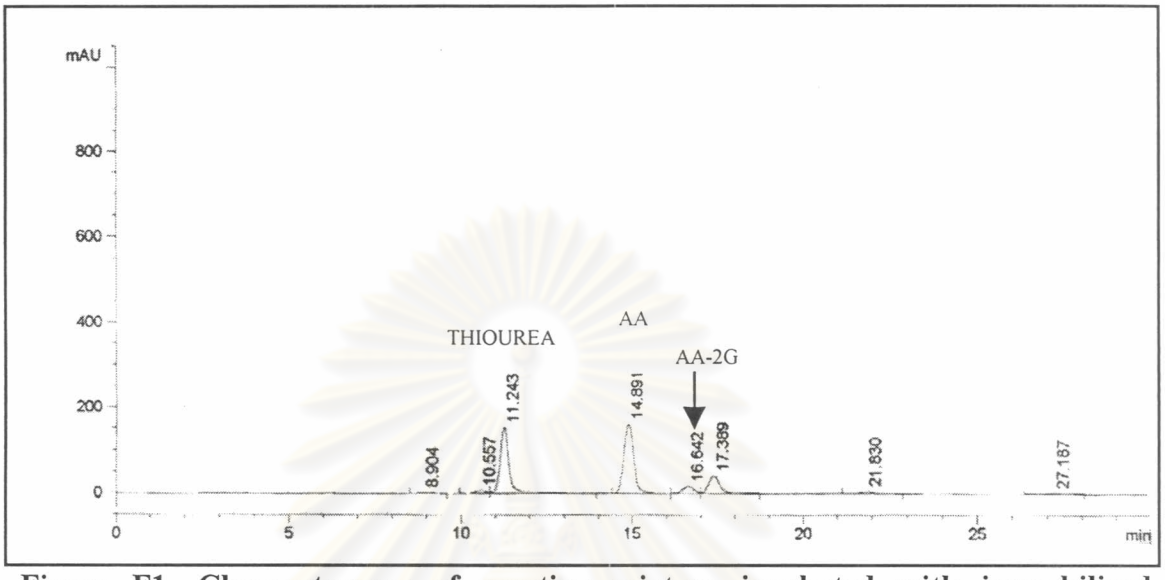


Figure F1. Chromatogram of reaction mixture incubated with immobilized CGTase (5 µl of the twenty-fold diluted sample was injected).

b) Washing solution

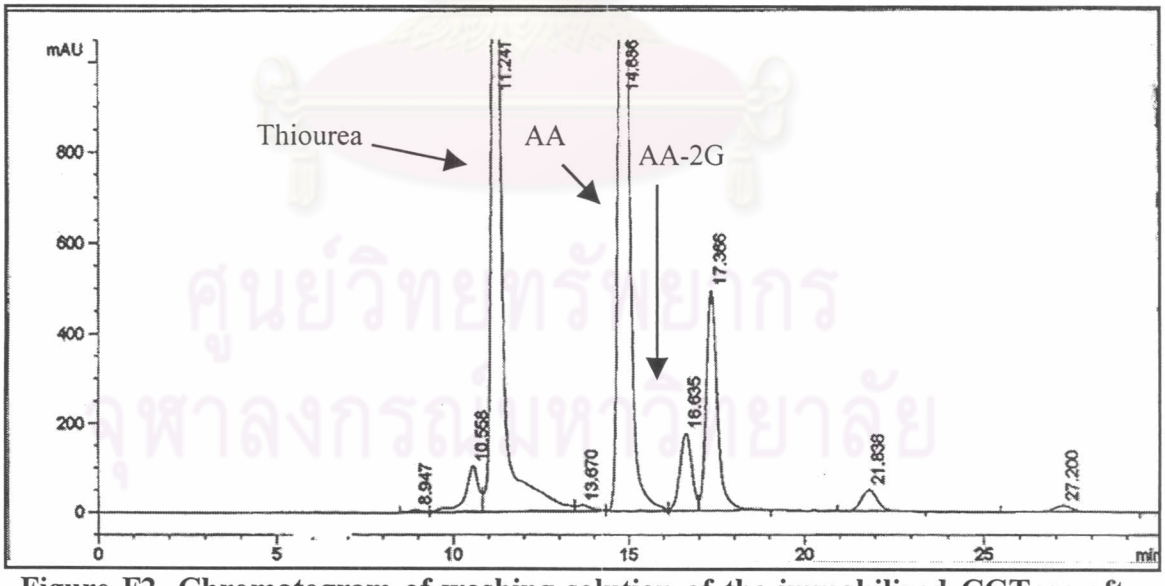


Figure F2. Chromatogram of washing solution of the immobilized CGTase after reaction (5 µl of the undiluted sample was injected).

Table F1 Amount of AA-2G and AA content recovered from immobilized CGTase after washing.

	AA content (g/l)	AA-2G content (g/l)
Reaction mixture	2.005	0.432
Washing solution no. 1	1.035	0.213

BIOGRAPHY

Miss Supranee Pantatan was born on February 16, 1977. She graduated with the Bachelor Degree of Science in Chemistry from Silapakorn University in 1999 and studying for Master in Biochemistry Program, Faculty of Science, Chulalongkorn University.