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APPENDICES

APPENDIX 1: Preparation for polyacrylamide gel electrophoresis

1) Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100ml

acrylamide	29.2 g
N, N′-methylene-bis-acrylamide	0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	18.17 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

2 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	24.2 g

Adjusted pH to 8.8 with 1M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	6.06 g

Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	12.1 g

Adjusted pH to 6.8 with 1M HCl and adjusted volume to 100 ml with distilled water

Solution B (SDS PAGE)

2 M Tris-HCl pH 8.8	75 ml
10% SDS	4 ml
distilled water	21 ml

Solution C (SDS PAGE)

1 M Tris-HCl pH 6.8	50 ml
10% SDS	4 ml
distilled water	46 ml

2. Non-denaturing PAGE

7.5% Separating gel

30% acrylamide solution	2.5 ml
1.5 M Tris-HCl pH 8.8	2.5 ml
distilled water	5.0 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 µl

5.0% stacking gel

30% acrylamide solution	0.67 ml
0.5 M Tris-HCl pH 6.8	1.0 ml
distilled water	2.3 ml
10% $(NH_4)_2S_2O_8$	30 µl
TEMED	5 µl

Sample buffer

1 M Tris-HCl pH 6.8	3.1 ml
glycerol	5.0 ml
1% bromophenol blue	0.5 ml
distilled water	1.4 ml

One part of sample buffer was added to four parts of sample.

Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g

Dissolved in distilled water to 1litre (final pH should be 8.8).

3. SDS-PAGE

7.5% separating gel

30% acrylamide solution	2.5 ml
solution B	2.5 ml
distilled water	5.0 ml
10% $(NH_4)_2S_2O_8$	50 µl
TEMED	10 µl

5.0% stacking gel

30% acrylamide solution	0.67 ml
solution C	1.0 ml
distilled water	2.3 ml
10% $(NH_4)_2S_2O_8$	30 µl
TEMED	5 µl

Sample buffer

1 M Tris-HCl pH 6.8	0.6 ml
50% glycerol	5.0 ml
10% SDS	2.0 ml
2-mercaptoethanol	0.5 ml
1% bromophenol blue	1.0 ml
distilled water	0.9 ml

One part of sample buffer was added to four parts of sample. The mixture was heated 5 minutes in boiling water before loading to the gel.

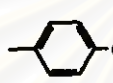
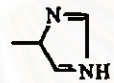
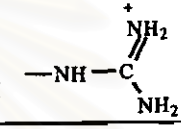
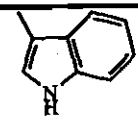
Electrophoresis buffer, 1 litre

Tris (hydroxymethyl)-aminomethane	3.0 g
Glycine	14.4 g
SDS	1.0 g

Adjusted volume to 1litre with distilled water

(pH should be approximately 8.3).

Appendix 2 Reactivities of amino acid side chains (continued)

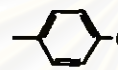
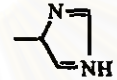
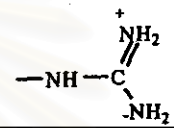
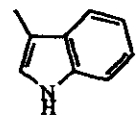
Reagent	—NH_2	—SH				—COOH		—S—S—	—S—CH_3
Phenylglyoxal	++	-	-	-	+++	-	-	-	-
Photooxidation	-	+++	±	+++	-	-	+++	±	+++
Sodium borohydride	-	+++[b]	++[b]	++[b]	-	-	-	-	-
Succinic anhydride	+++	+++	-	-	-	-	+++	-	-
Sulfite	-	+++	+++	+++	-	-	-	-	-
Sulfonyl halides	+++	+++	+++	-	-	-	+	-	+
Tetranitromethane	-	+++	+++	-	-	-	+	-	+
Tetrathionate	-	+++	-	-	-	-	-	-	-
Thiols	-	-	-	-	-	-	-	+++	-
Trinitrobenzenesulfonic acid	+++	++[b]	-	-	-	-	-	-	-
Water-soluble carbodiimide and nucleophile	±	±	±	-	-	+++	-	-	-

[a] -, +, ++, and +++ indicate relative reactivities; ±, ±±, and ±±± likewise indicate relative reactivities which may or may not be attained depending on the condition used.

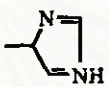
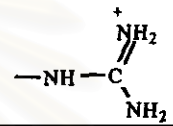
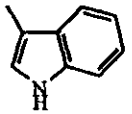
[b] Spontaneously reversible under the reaction conditions or upon dilution, regenerating original group.

[c] Easily reversible, regenerating original group.

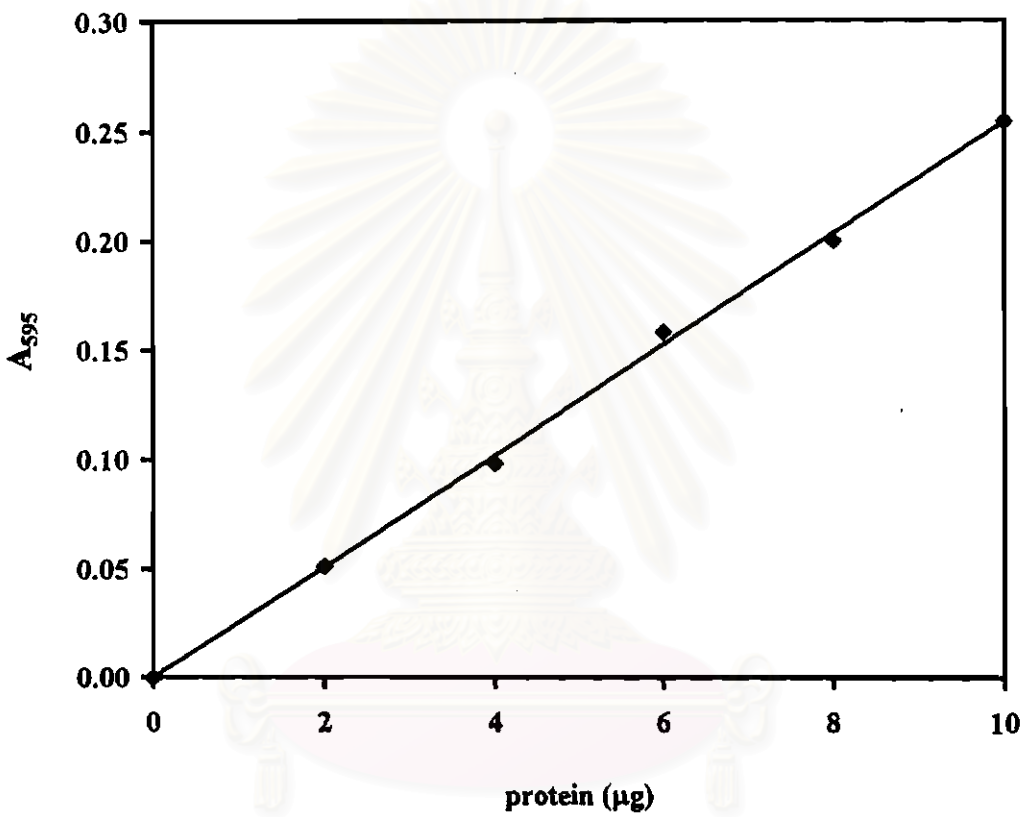
Appendix 2 Reactivities of amino acid side chains (continued)

Reagent	—NH_2	—SH				—COOH		—S—S—	—S—CH_3
N-ethylmaleimide	±	+++	-	-	-	-	-	-	-
Ethyl thiotrifluoacetate	+++[b]	-	-	-	-	-	-	-	-
Formaldehyde	+++	+++	+++	+++	+	-	+	-	-
glyoxal	++	-	-	-	+++	-	-	-	-
Haloacetates	+	+++	-	+	-	-	-	-	+
Hydrogen peroxide	-	+++	-	-	-	-	+	+	+++
2-hydroxy-5-nitrobenzyl bromide	-	++	-	-	-	-	+++	-	-
Iodine	-	+++	+++	+++	-	-	-	-	-
0-iodosobenzoate	-	+++	-	-	-	-	-	-	-
Maleic anhydride	+++[c]	++[c]	++[b]	++[b]	-	-	-	-	-
p-mercuribenzoate	-	+++	-	-	-	-	-	-	-
Methanol/ HCl	-	-	-	-	-	+++	-	-	-
2-methoxy-5-nitrotropone	+++[c]	-	-	-	-	-	-	-	-
Methyl acetimidate	+++	-	-	-	-	-	-	-	-
O-methylisourea	+++	-	-	-	-	-	-	-	-
Nitrous acid	+++	+++	±	-	-	-	-	+	-
Performic acid	-	+++	-	-	-	-	++	+++	+++

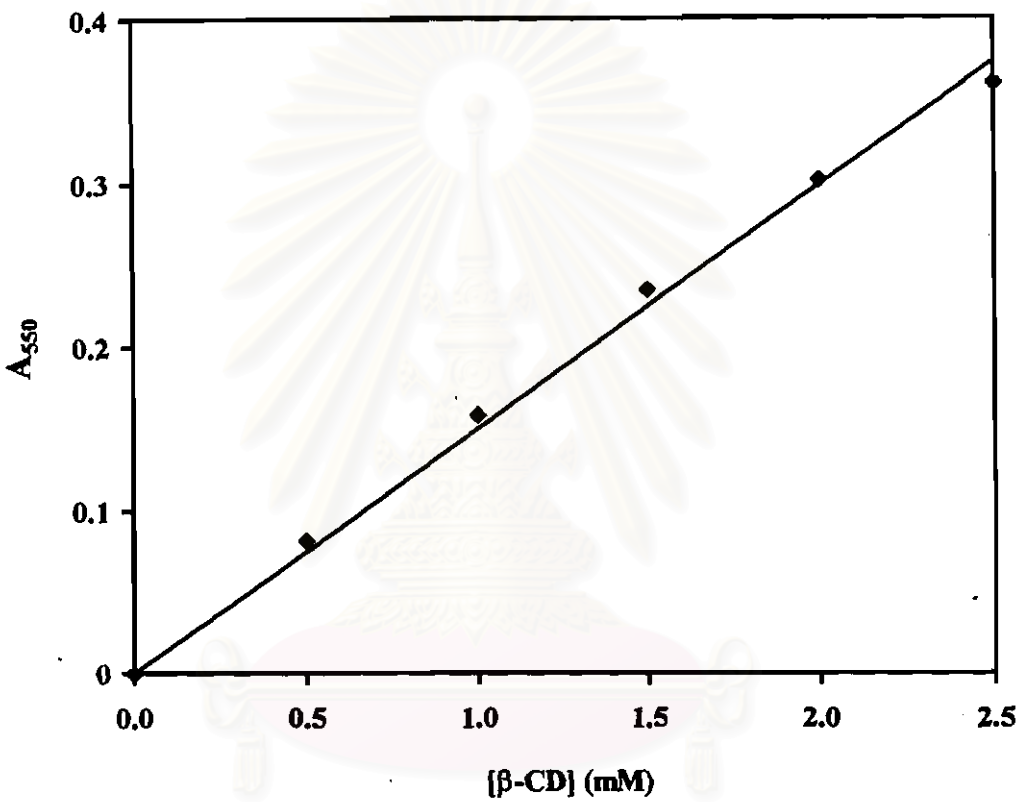
Appendix 2 Reactivities of amino acid side chains (Means and Feeney, 1971)

Reagent	—NH_2	—SH				—COOH		—S—S—	—S—CH_3
Acetic anhydride	+++	+++[b]	+++[c]	+++[b]	-	-	-	-	-
N-acetylimidazole	++	+++[b]	+++[c]	+++[b]	-	-	-	-	-
acrylonitrile	++	+++	-	-	-	-	-	-	-
Aldehyde/ $NaBH_4$	+++	-	-	-	-	-	-	-	-
N-bromosuccinimide	-	+++	++	+	-	-	+++	-	-
N-carboxyanhydrides	+++	-	-	-	-	-	-	-	-
Cyanate	+++	+++[b]	++[b]	+[b]	-	+[b]	-	-	-
Cyanogen bromide	-	+	-	-	-	-	-	-	+++
1,2-cyclohexanedione	±	-	-	-	+++	-	-	-	-
Diacetyl trimer	+	-	-	-	+++	-	-	-	-
Diazoacetates	-	++	-	-	-	+++	-	-	-
Diazonium salts	+++	+	+++	+++	+	-	+	-	-
Diethylpyrocarbonatr	+++	-	-	+++[c]	-	-	-	-	-
Diketone	+++[c]	-	+	-	-	-	-	-	-
Dinitrofluorobenzene	+++	+++	++	++	-	-	-	-	-
5,5'-dithiobis (2-nitrobenzoic acid)	-	+++[c]	-	-	-	-	-	-	-
Ethyleneimine	-	+++	-	-	-	-	-	-	+

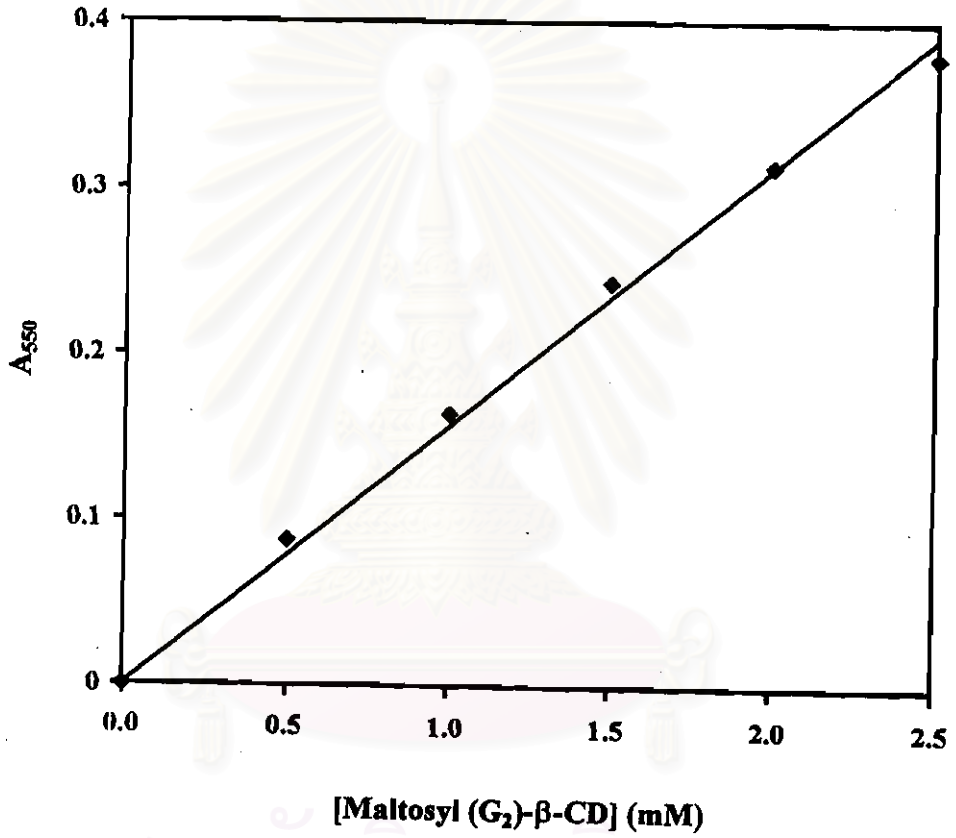
APPENDIX 3: Standard curve for protein determination by Bradford's method



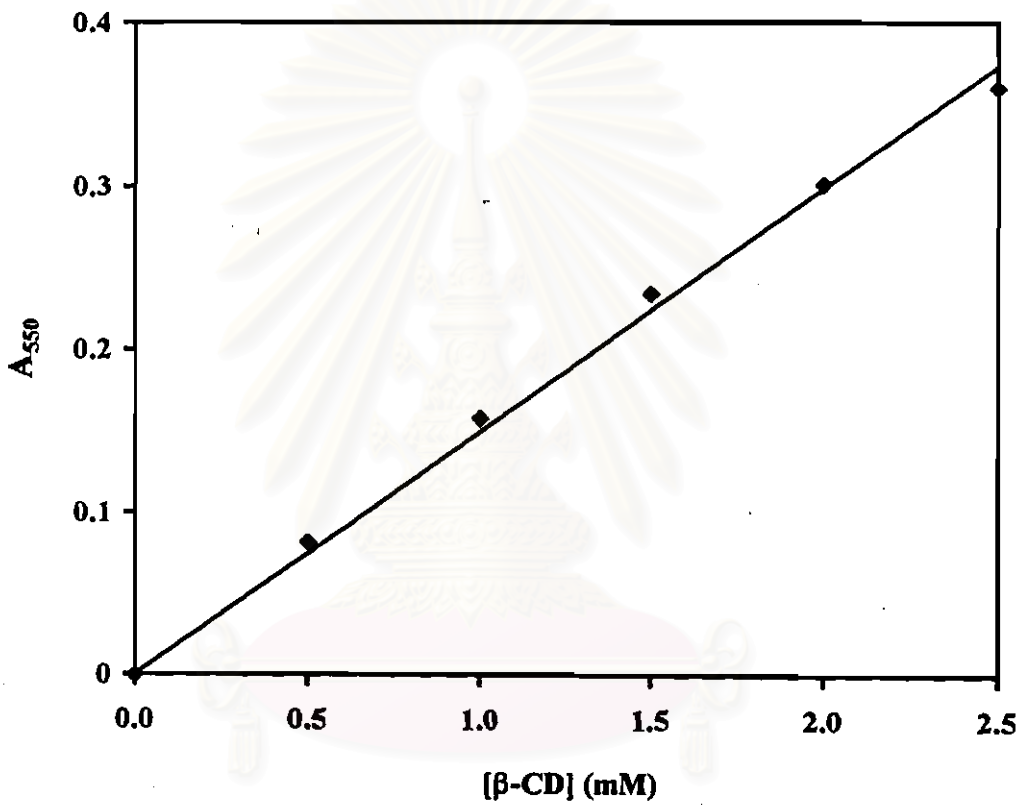
APPENDIX 4: Standard curve of β-cyclodextrin by phenolphthalein method



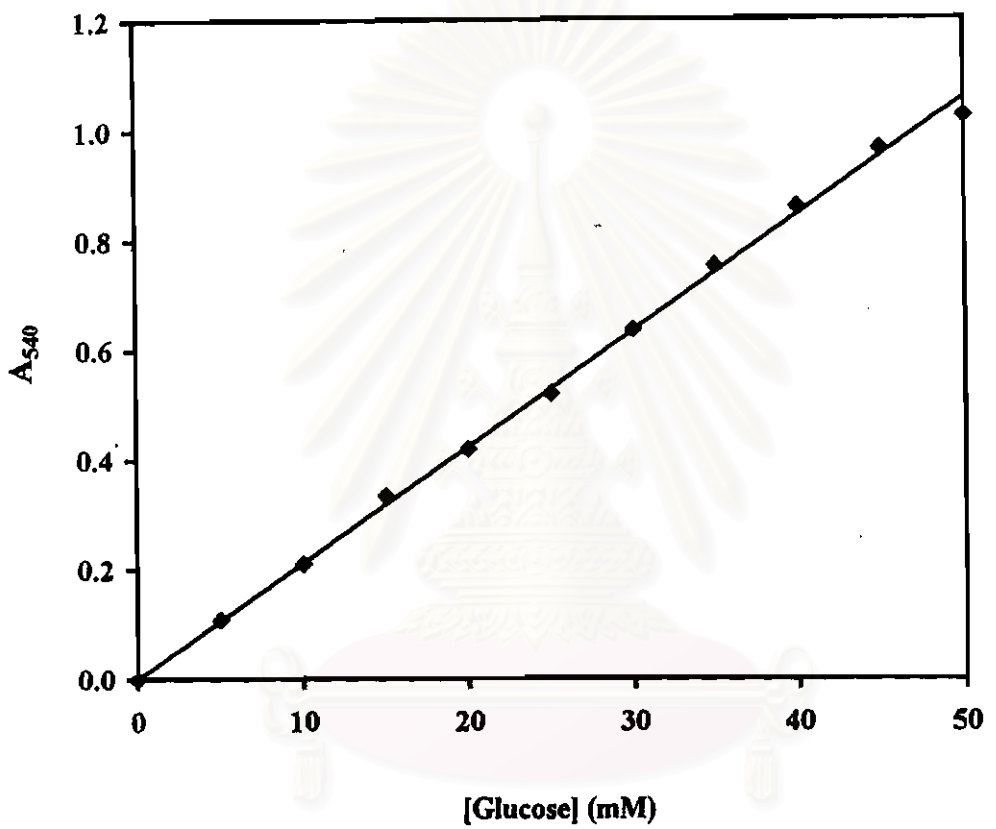
APPENDIX 5: Standard curve of maltosyl (G_2)-β-cyclodextrin by phenolphthalein method



APPENDIX 6: Standard curve of methyl-β-cyclodextrin by phenolphthalein method



APPENDIX 7: Standard curve of glucose by dinitrosalicylic acid method



BIOGRAPHY

Miss Anchalee Tongsima was born on May 3, 1973. She graduated with the Bachelor Degree of Science in Biochemistry from Chulalongkorn University in 1995 and continued studying for Master in Biochemistry Program.