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APPENDICES

APPENDIX A: Preparation for protein determination

Reagent for determination of protein concentration (modified from Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate, 1% potassium tartate, pH 7.0)

Potassium tartate	1 g
Copper sulfate	0.5 g
Adjust pH to 7.0 and adjust the solution volume to 100 ml.	

Solution B (2 % sodiumcarbonate, 1 N sodium hydroxide)

Sodium carbonate	20 g
Sodium hydroxide	4 g
Dissolved in 1,000 ml distilled water.	

Solution C (phenol reagent)

Sodium tungstate	50 g
Sodium molybdate	12.5 g
85 % phosphoric acid	25 ml
Distilled water	350 ml
Concentrated hydrochloric acid	50 ml
reflux for 10 hour	
Lithium sulphate	75 g
Distilled water	25 ml
Bromine solution	2-3 drops

Boiled the solution to reduce excess bromine for 15 min, adjusted volume to 500 ml with distilled water, stored at 4 °C. Diluted the stock solution with distilled water in ratio 1:1 (V/V) before using.

APPENDIX B: Polyacrylamide Gel Electrophoresis solution (Davis, 1964)

Solution A (0.2% TEMED (*N,N,N',N'*-tetramethylethylene diamine), 1.5 M Tris-HCl, pH 8.9)

Tris (Tris-hydroxymethyl aminomethane)	36.6 g
1 N HCl	48 ml
TEMED	0.23 ml

Adjusted pH to 8.9 by 1 N hydrochloric acid and adjusted volume to 100 with distilled water, the stock solution was stored at 4 °C.

Solution B (0.46 % TEMED and 0.5 M Tris-HCl, pH 6.7)

Tris-hydroxymethyl aminomethane	0.98 g
1 N HCl	48 ml
TEMED	0.4 ml

Adjusted pH to 6.7 with 1 N HCl and adjusted volume to 100 ml with distilled water. The solution was stored at 4°C.

Solution C (30.8 % Acrylamide)

Acrylamide	30 g
<i>N,N'</i> -methylene –bis-acrylamide	0.8 g

Adjusted volume to 100 ml with distilled water. The solution was stored at 4°C.

Solution D (12.5 % Acrylamide)

Acrylamide	10 g
Bis	2.5 g

Adjusted volume to 100 ml with distilled water. The solution was stored at 4°C.

Solution E (0.004 % riboflavin)

Riboflavin	0.004 g
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Adjusted volume to 100 ml with distilled water and stored in the dark, at 4°C.

Solution F(0.05 M Tris and 0.384 M glycine; 10 fold concentrated)

Tris	10 g
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Glycine	28.8 g
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Adjusted volume to 1,000 ml with distilled water and stored in the 4°C.

Diluted with distilled water 10 fold before using.

Solution G (40 % sucrose)

Sucrose	40 g
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Adjusted volume to 100 ml with distilled water, stored at - 20°C.

Dye marker (0.05 % Bromophenol Blue, 40 % sucrose)

Bromophenol Blue	5 mg
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Sucrose	4 g
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Distilled water	10 ml
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Protein staining solution (0.44 % Coomassie Brilliant Blue G-250 and 3.5 % perchloric acid)

Coomassie Brilliant Blue G-250	0.28 g
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Perchloric acid	4.5 ml
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Distilled water	675.5 ml
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Destaining solution (7 % acetic acid)

Glacial acetic acid	35 ml
Distilled water	500 ml

Enzyme activity staining solution

Tris-HCl, pH 8.5	4.25 mM
L-alanine	40 μ M
NAD ⁺	50 μ M
Phenazine methosulfate	250 μ g
Nitroblue tetrazolium	2.5 mg
Distilled water	10 ml



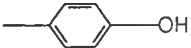

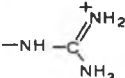

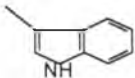

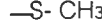
APPENDIX C: Preparation for HPLC chromatography**The solution in HPLC chromatography****Solution A (0.1 % Trifluoroacetic acid (TFA) in ultrapure water)**

99.9 % TFA	999 μ l
Ultrapure water	1,000 ml

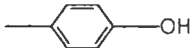
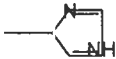
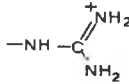
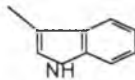
Solution B (0.07% TFA acetonitrile HPLC grade)

99.9% TFA	700 μ l
Acetonitrile	999 ml



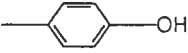
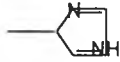
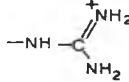
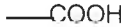
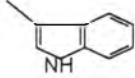

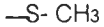
APPENDIX D: Reactivity of amino acid side chains (Means and Feeney, 1971)

Reagent									
Acetic anhydride	+++	+++ ^b	+++ ^c	+++ ^b	-	-	-	-	-
<i>N</i> -acetylimidazole	++/-	+++ ^b	+++ ^c	+++ ^b	-	-	-	-	-
Acrylonitrile	++/-	+++	-	-	-	-	-	-	-
Aldehyde/ NaBH_4	+++	-	-	-	-	-	-	-	-
<i>N</i> -bromosuccinimide	-	+++	++	+	-	-	+++	-	-
<i>N</i> -carboxyanhydride	+++	-	-	-	-	-	-	-	-
Cyanate	+++	+++ ^b	++ ^b	+ ^b	-	+ ^b	-	-	-
Cyanogen bromide	-	+	-	-	-	-	-	-	+++
1,2-cyclohexanedione	+/-	-	-	-	+++	-	-	-	-

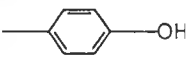
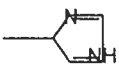
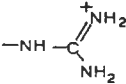
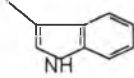
APPENDIX D: Reactivity of amino acid side chains (continued)

Reagent	—NH	—SH				—COOH		—S—S—	—S—CH_3
Diacetyl trimer	+	-	-	-	+++	+	-	+	-
Diazoacetates	-	++	-	-	-	+++	-	-	-
Diazonium salts	+++	+	+++	+++	+	-	+	-	-
Diethylpyrocarbonate	+++	-	-	+++ ^c	-	-	-	-	-
Diketone	+++ ^c	-	+	-	-	-	-	-	-
Dinitrofluorobenzene	+++	+++	++	++	-	-	-	-	-
5,5'-dithiobis(2-nitrobenzoic acid)	-	+++ ^c	-	-	-	-	-	-	-
Ethyleneimine	-	+++	-	-	-	-	-	-	+
N-ethylmaleimide	++/-	+++	-	-	-	-	-	-	-
Ethyl thiotrifluoroacetate	+++ ^b	-	-	-	-	-	-	-	-



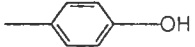
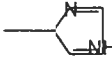
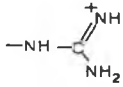

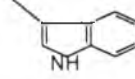

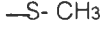
APPENDIX D: Reactivity of amino acid side chains (continued)

Reagent	 —NH	 —SH				 —COOH		 —S-S—	 —S-CH ₃
Formaldehyde	+++	+++	+++	+++	+	-	+	-	-
Glyoxal	++	-	-	-	+++	-	-	-	-
Haloacetates	+	+++	-	+	-	-	-	-	+
Hydrogen peroxide	-	+++	-	-	-	-	+	+	+++
2-hydroxy-5-nitrobenzyl bromide	-	++	-	-	-	-	+++	-	-
Iodine	-	+++	+++	+++	-	-	-	-	-
O-iodosobenzoate	-	+++	-	-	-	-	-	-	-
Maleic anhydride	+++ ^c	++ ^c	++ ^b	++ ^b	-	-	-	-	-
<i>p</i> -mercuribenzoate	-	+++	-	-	-	-	-	-	-
Methanol/HCl	-	-	-	-	-	+++	-	-	-
2-methoxy-5nitropropane	+++ ^c	-	-	-	-	-	-	-	-

APPENDIX D: Reactivity of amino acid side chains (continued)

Reagent	—NH	—SH				—COOH		—S—S—	—S—CH_3
Methyl acetimidate	+++	-	-	-	-	-	-	-	-
O-methylisourea	+++	-	-	-	-	-	-	-	-
Nitrous acid	+++	+++	+/-	-	-	-	-	+	-
Performic acid	-	+++	-	-	-	-	++	+++	+++
Phenylglyoxal	++	-	-	-	+++	-	-	-	-
Photooxidation	-	+++	++/-	+++	-	-	+++	+/-	+++
Sodium borohydride	-	+++ ^b	++ ^b	++ ^b	-	-	-	-	-
Succinic anhydride	+++	+++	-	-	-	-	+++	-	-
Sulfite	-	+++	+++	+++	-	-	-	-	-
Sulfonyl halides	+++	+++	+++	-	-	-	+	-	+
Tetraiomethane	-	+++	+++	-	-	-	+	-	+

APPENDEX D: Reactivity of amino acid side chains (continued)

Reagent									
Tetrathionate	-	+++	-	-	-	-	-	-	-
Thiols	-	-	-	-	-	-	-	+++	-
2,4,6-Trinitrobenzenesulfonic acid	+++	++ ^b	-	-	-	-	-	-	-

Symbol; +++ = highly reactive, extensive reaction under typical condition

++ = the significant reaction should be expected

+ = some reaction is possible, but is not usually extensive

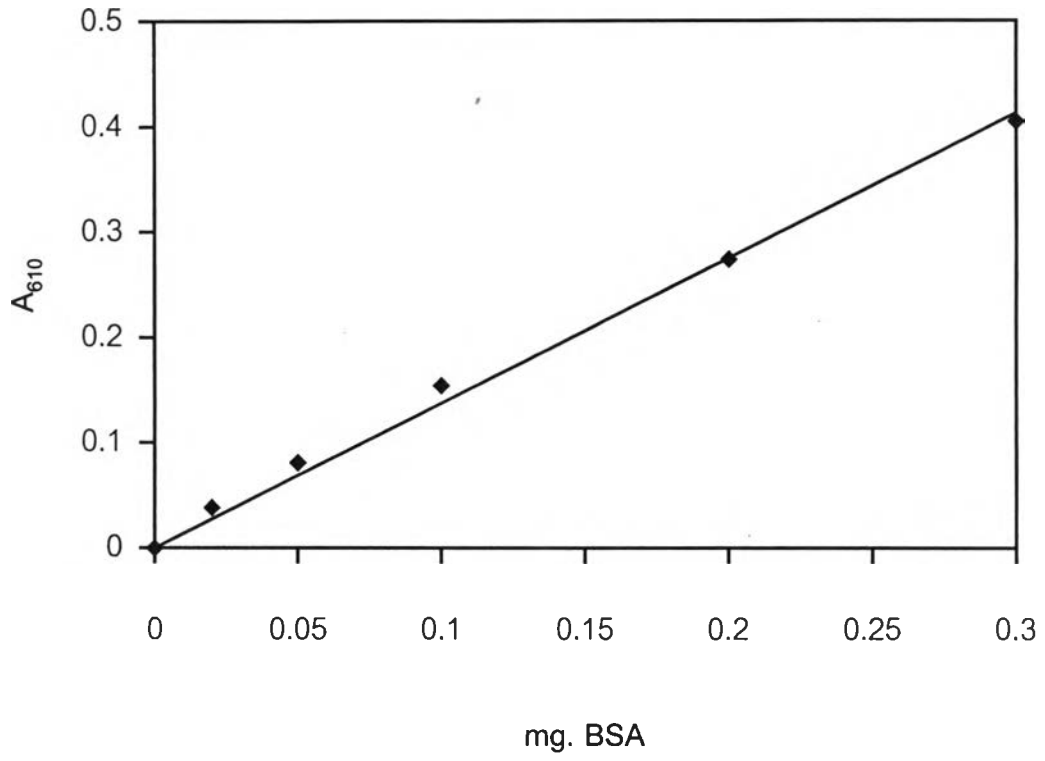
- = no reaction expected under typical condition

+++/--, ++/-- and +/- = used similarly to indicate the expected reactivities in case where resulting derivatives are unstable and spontaneously revert under normal condition or upon dilution or dialysis, to the unmodified side-chain.

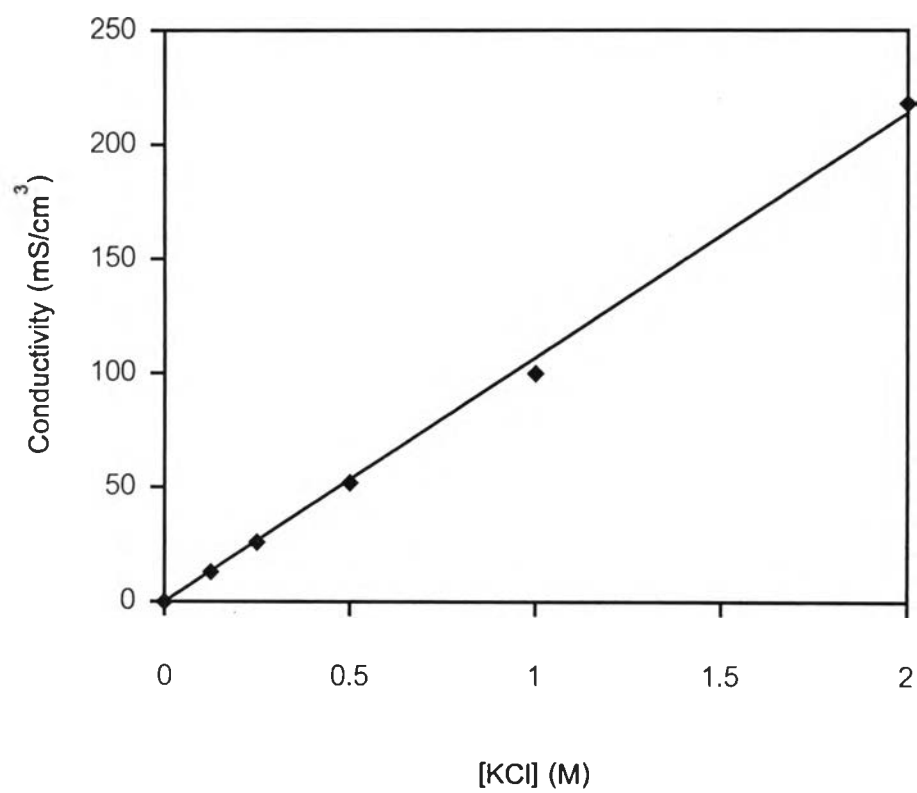
^b Spontaneously reversible under the reaction condition or upon dilution, regenerating original group.

^c Easily reversible, regenerating original group.

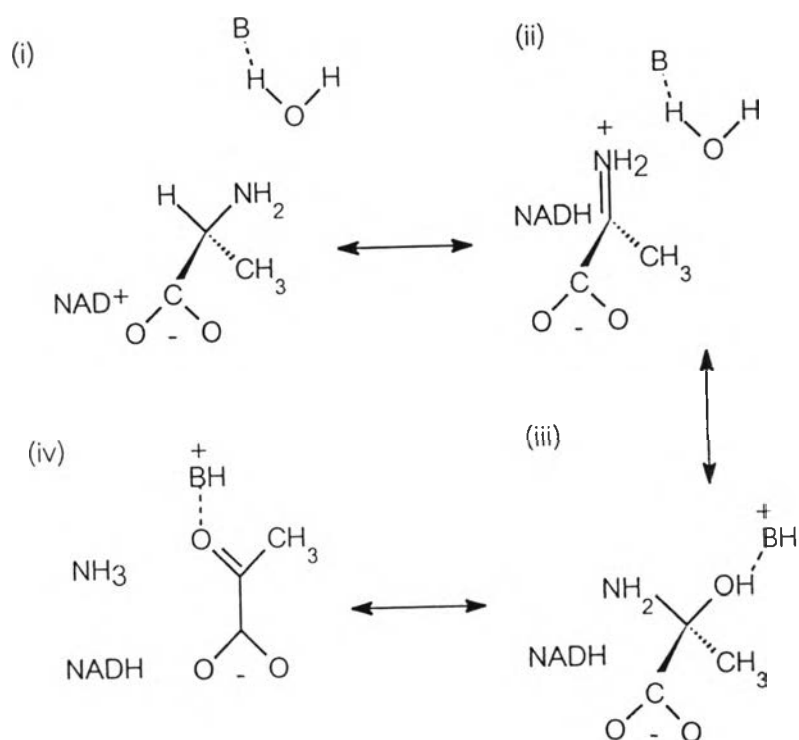
APPENDIX E : Standard curve for protein determination by Lowry ' s method



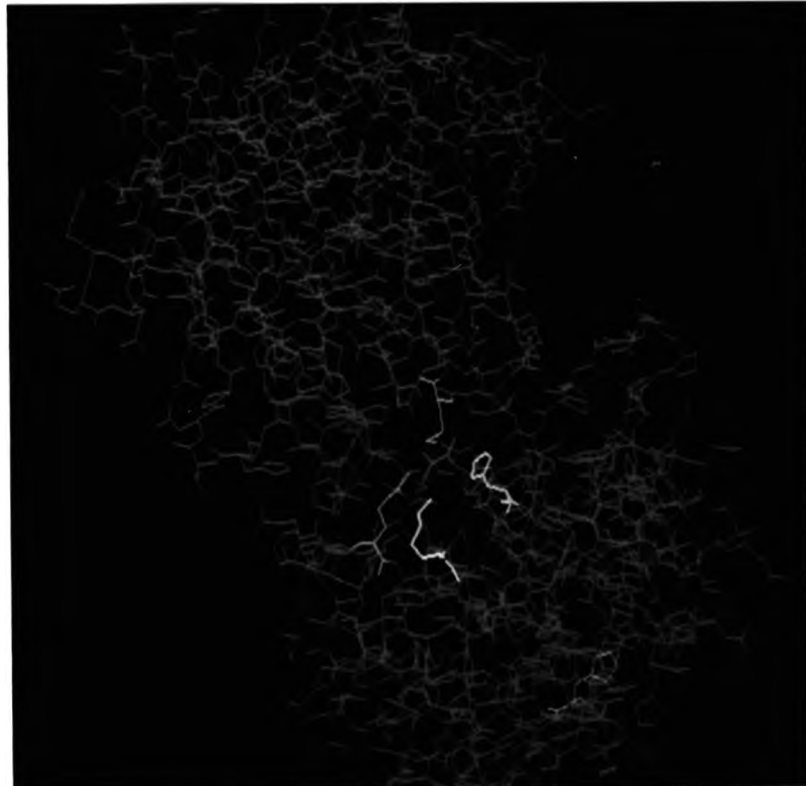
APPENDIX F Standard curve of conductivity of potassium chloride



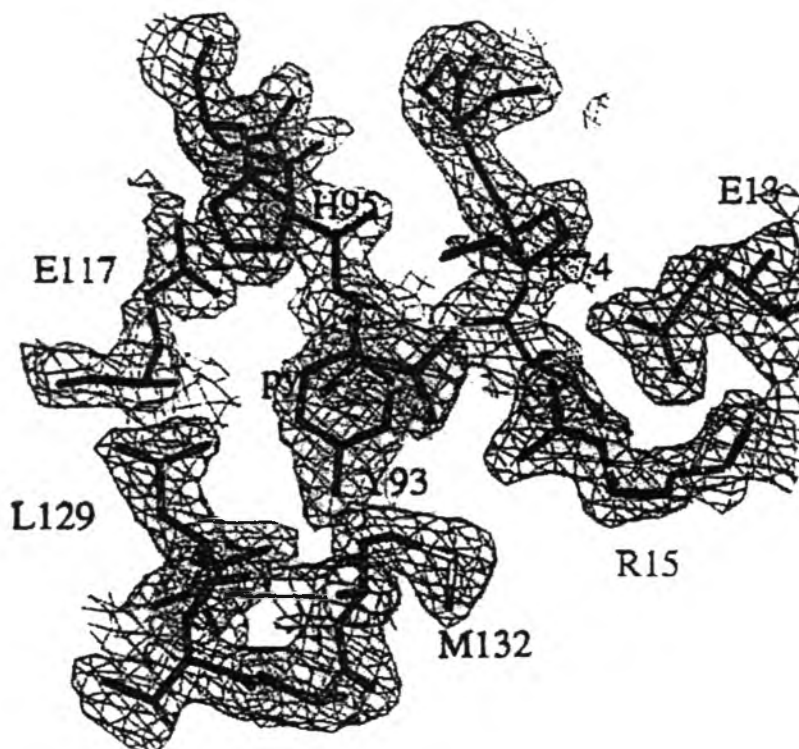
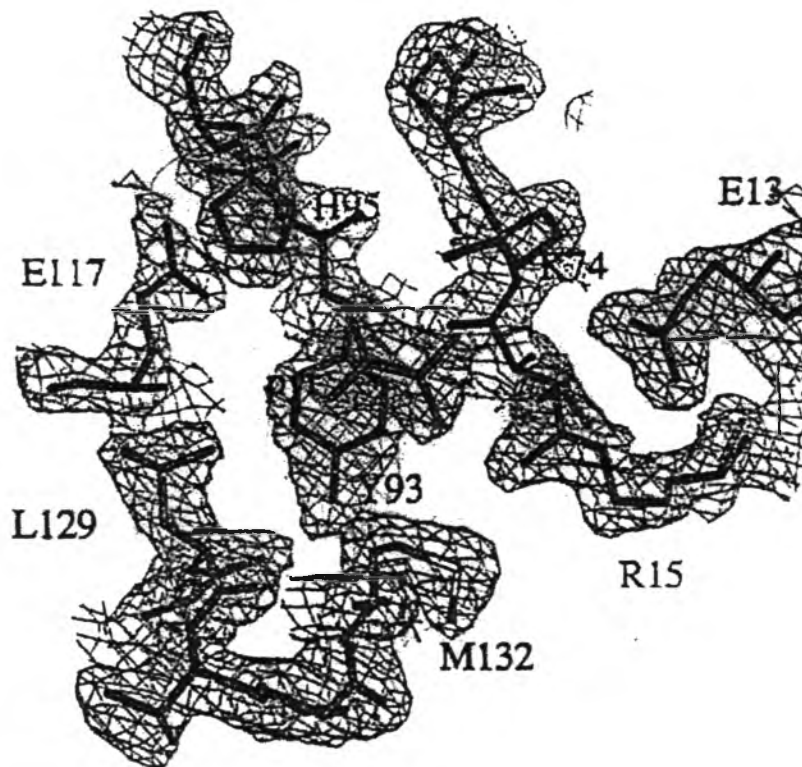
APPENDIX G: The proposed intermediates of the alanine dehydrogenase catalytic cycle. In panel (i) the hydride transfer from the C α of the alanine to the C4 of the nicotinamide ring leads to the iminopyruvate shown in panel (ii). The ensuing attack by a water molecule, facilitated by the base, B, results in the formation of the cabinolamine shown in panel (iii). Subsequence collapse of the intermediate yield pyruvate and ammonia panel (iv). (Baker *et al.*, 1998)



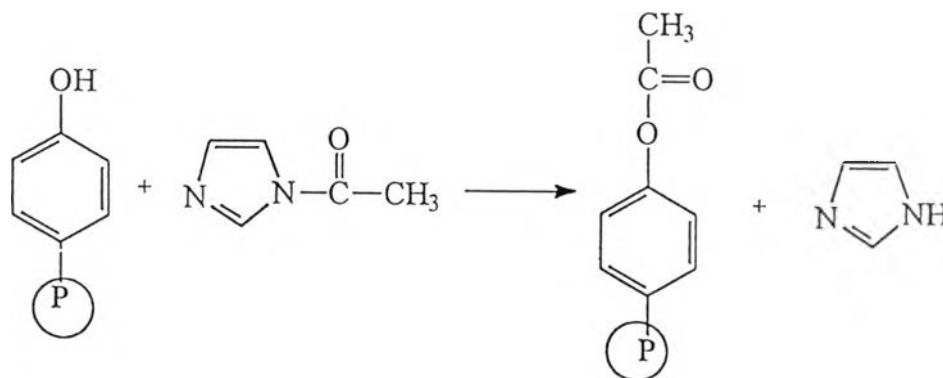
APPENDIX H: Three-dimensional structure of alanine dehydrogenase from *Phormidium lapidium* with pyruvate packed against the enzyme: pyruvate, red ; arginine-15, pink; lysine-74, white ; histidine-95, yellow ; methionine-132, green (Baker *et al.*,1998)



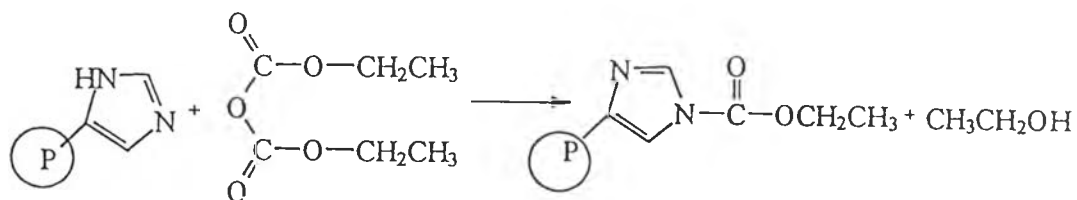
APPENDIX I: Stereodiagram of the pyruvate binding site in the binary complex of alanine dehydrogenase with pyruvate (Baker *et al.*, 1998)



APPENDIX J: Modification reaction of group-specific reagents

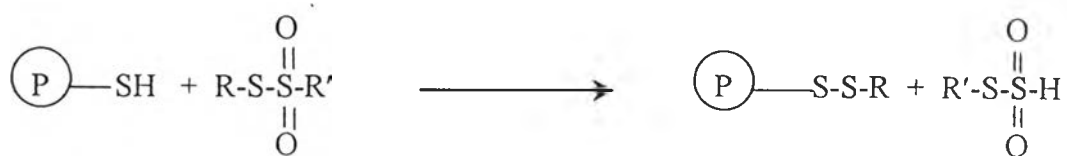


Reaction 1 Modification reaction of NAI with tyrosine residue in protein (P) (Means and Feeney, 1971)

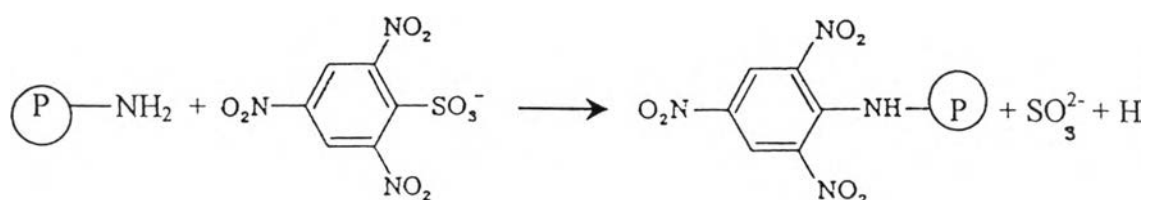


Reaction 2 Modification reaction of DEPC with histidine residue in protein (P) (Means and Feeney, 1971)

APPENDIX J: Modification reaction of group-specific reagents (continued)



Reaction 3 Modification reaction of DTT with cysteine residue in protein (P) (Means and Feeney, 1971)



Reaction 4 Modification reaction of TNBS with lysine residue in protein (P) (Means and Feeney, 1971)

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