

CHAPTER II



MATERIALS

2.1 Materials

Whatman Filter Paper No. 1 and Whatman Chromatographic Paper 3 MM were products of Whatman Ltd., England.

Micro-hematocrit capillary tubes were products of Fisher Scientific Co., Pittsburgh, U.S.A.

Scintillation vials were products of Packard Instruments Co., U.S.A.

PM-10 Membranes for diaflo ultrafiltration were products of Amicon Corporation, Lexington, Massachusetts, U.S.A.

2.2 Microorganism

Escherichia coli strain K12 ATCC 3110 was used throughout the work.

2.3 Chemicals

Ammonium persulfate (AR grade), naphthalene (scintillation grade), cyanuric chloride (2,4,6-trichloro-1,3,5-triazine), and toluene (AR grade) were products of E. Merck, Damstadt, Germany.

Coomassie brilliant blue G-250, dithiothreitol (DTT) (puriss biochim grade), 4-ethylmorpholine (purum grade), bis-(3-aminopropyl) amine (purum grade), sulfanilamide (puriss grade), iodoacetamide (puriss grade), 2,3-butanedione (puriss grade), 2-ethoxy-ethanol (purum grade), tris (hydroxymethyl) aminomethane (puriss grade), di-potassium hydrogenphosphate (purum grade), sodium carbonate (purum grade) and dimethylformamide (puriss grade) were products of Fluka AG, Buchs, Switzerland.

Sepharose 4B was product of Pharmacia Fine Chemicals AB Sweden.

N,N'-Methylenebisacrylamide, acrylamide, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (Hepes), bovine serum albumin (fraction V, A-grade, crystallized), 2,5-diphenyloxazole (PPO) (scintillation grade), 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) (scintillation grade), p-chloromercuribenzoic acid, phenylmethylsulfonyl fluoride, phenylglyoxal hydrate, 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulfonate, sucrose, and 2,4,6-trinitrobenzenesulfonate were products of Sigma Chemical Company, St. Louis, U.S.A.

N,N,N',N'-Tetramethylethylenediamine, 2,4-pentanedione, cupric sulfate, coomassie brilliant blue (for electrophoresis), ethylene glycol (extra pure grade), sodium dithionite, magnesium sulfate (AR grade), glycine, 2-mercaptoethanol, sodium-potassium tartrate, and riboflavin were products of BDH Chemical Ltd. Poole, England.

Aquacide II A was product of Calbiochem, San Diego, California, U.S.A.

Toluene was product of Ajax Chemical, Sydney, Australia.

Agar and tryptone were products of Difco Laboratories, Detroit, Michigan, U.S.A.

Yeast extract was product of BBL, Cockeysville, Maryland, U.S.A.

Glacial acetic acid (AR grade) was product of Carlo Erba, Division Chemical, Milano, Italy.

¹⁴C-p-Aminobenzoic-7-¹⁴C-acid (0.1 mCi/17.25 μmol) was product of New England Nuclear, Boston, Massachusetts, U.S.A.

Ammonium sulfate (AR grade), hydrochloric acid (AR grade), and sodium hydroxide (AR grade) were products of J.T. Baker Chemical B.V. Deventor, Holland.

Potassium di-hydrogen phosphate, boric acid, dioxane, magnesium chloride, potassium chloride, and sodium hydrogen carbonate were products of May and Baker Ltd., Bagenham, England.

Sodium chloride and methanol were products of Mallinckrodt Inc., Kentucky, U.S.A.

Urea was product of Riedel-DeHaen AG, Germany.

The following substances were kindly supplied by Mr. Jirasak Kongkiattikajorn. The references for the preparation of these substances were indicated in parenthesis. The substances were : 4-(4-aminobenzenesulfonamido) benzenesulfonylglycine (Kolloff, 1938; Bauer, 1939), 2-amino-4-hydroxy-6-hydroxymethylpteridine pyrophosphate (Ho et al. 1974a), N-(p-aminobenzenesulfonyl) glycine (Kolloff, 1938), N-(p-aminobenzenesulfonyl) alanine (modified from the method of Archer, Hoppe, Lewis, and Hoskell, 1951), N-(p-aminobenzenesulfonyl) methionine (modified from the method of Archer et al. 1951 and the method of

Giannini and Gallice, 1956), N-(p-aminobenzenesulfonyl) valine (Archer et al. 1951), N-(p-aminobenzenesulfonyl) leucine (Archer et al. 1951), N-(p-aminobenzenesulfonyl) tyrosine (modified from the method of Archer et al. 1951 and the method of Giannini et al. 1956), and N-(p-aminobenzenesulfonyl) phenylalanine (Archer et al. 1951).

Phenol reagent (Folin-Ciocalteu) was prepared by Mrs. Somporn Sattayasunsakun and Mr. Sopon Pinpet in our laboratory using the method of Folin and Ciocalteu (1927).

2.4 Equipments

Refrigerated Centrifuge, model J. 21C (Beckman Instruments Inc., Fullerton, California, U.S.A.);

Fermentation Equipment, model MD500-10L (Marubishi Laboratory Equipment Co., Ltd. Japan);

Sonicator, model W375 (Heat System-Ultrasonic Inc., New York, U.S.A.);

Ultrarack Fraction Collector, model 2070 (LKB-Productor Ab. Stockholm 12, Sweden);

Automatic pipet : Pipetman (Gilson Medical Electronics, France);

Fisher-Johns Melting Point Apparatus (Fisher Scientific Co., U.S.A.);

Liquid Scintillation Counter (Packard Instrument Co., U.S.A.);

Bio-Freezer (Forma Scientific, Marietta, Ohio, U.S.A.);

Autocal pH Meter, model PHM 83 Laboratory (Radiometer, Copenhagen, Denmark);

Analytical Balance, model 2355 (Sartorius Werke, Gottingen, Germany);

Spectrophotometer, model Spectronic 20 (Bausch & Lomb, U.S.A.);

Spectrophotometer, model 25 (Beckman Instruments Inc., Fullerton, California, U.S.A.);

Autoclave, model HA-3D (Hirayama Manufacturing Co., Tokyo, Japan);

Water bath, model BKM 250 (Gallenkamp & Co., Ltd. London, England);

Shaker bath, Model 2563 (Forma Scientific, Marietta, Ohio, U.S.A.);

Shaker bath, type 01 PF 623, No 8108 (Heto Lab Equipment, Denmark);

HSI disc electrophoresis chamber, model DE 102 (Haefer Scientific Instruments, San Francisco, U.S.A.);

Shandon Power supply, model Vokam 254 (Shandon Scientific Co., Ltd., England);

Diaflo Ultrafiltration (Amicon Corporation, Lexington, Massachusetts, U.S.A.);

Conductometer, model RC 216 B2 (Beckman Instruments Inc., Fullerton, California, U.S.A.).