Materials and Methods

Chemicals

Acrylamide (electrophoresis grade), AR grade captopril, *L*-cysteine, glutathione, Ellman's reagent, (ESSE), sodium chloride, Tris.HCl, potassium hydrogen phthalate, potassium dihydrogen phosphate, ascorbic acid (H_2ASc), and HPLC grade methanol were purchased from Aldrich (Fairlawn, NJ). Deionised water was prepared in house using a Labconco Water Pro PS deionizer (Labconco Water Corporation, Kansas City, MO). All stock and working solutions were prepared in deionised water. HCl (Analar grade) was obtained from BDH Laboratories (Poole, England).

Spectrophotometric Measurements

The acrylamide-thiol (AA-SR) complexes were monitored spectrophotometrically on a HP 8453 Diode Array Spectrophotometer with 1 cm path length quartz cuvettes. The wavelengths monitored were 327 nm for ESSE and 409 nm for the Ellman's thiolate (ES^-) anion. The diode array spectrophotometer was operated in the kinetic mode for determination of reaction parameters and in the standard mode for quantifying the rate constants associated with each AA-thiol reaction. Temperature was maintained by a Lauda-Brinkmann RM6 B Ciculator/Thermostated Water Bath (Lauda Dr. R. Wobser, GMBH and Co., Lauda-Königshofen, Germany).

In the kinetic mode, all reactions were conducted at 310 K. In the standard mode with varied AA concentrations, the reactions were conducted at 310 K. For varied thiol concentrations, the reactions were conducted at 293, 298, 303 K for CySH and 303, 310, and 315 K for CapSH and GSH. Mixtures containing CapSH and GSH were measured at 1 minute

intervals while CySH mixtures were measured at 30 s intervals. The pH of the final solutions were recorded using an Orion Research Expandable ion Analyzer EA 920 (Orion Research Inc., Boston, M.A., U.S.A.) calibrated with buffers: 4.01 (0.0496 mol dm^{-3} $K_2HC_8H_4O_4$) and 6.87 (0.025 mol dm^{-3} KH_2PO_4).

LC/DAD/MS Analysis

The AA–SR complexes were analyzed on an Agilent 1100 Series LC coupled to an Agilent 1100 Series MS Detector (Agilent, Technologies, Palo Alto, CA, U.S.A.). Separation of the complexes were achieved using a Zorbax Eclipse XDB–C18 column (150 mm x 4.6 mm ID x 5 μm particle size; Agilent Technologies, Palo Alto, CA, U.S.A.) with a Zorbax XDB–C18 guard column (12.5 mm x 4.6 mm i.d.) at a flow rate of 0.2 mL/min; Operating parameters were: positive electrospray ionization (+ESI) in the SIM and scan modes; SIM ions: 308, 379, 380, 613; scan range: 300 – 400 amu (for the AA–SG complex); Mobile phase: methanol/water (5 + 95 v/v)%; run time 8.00 min; MS: Drying gas temperature: 350 ^{o}C; drying gas flow: 12 L/min; nebulizer gas pressure: 35 psig; capillary voltage: 3000 V.; Fragmentor voltage: 80V (scan), 70V (SIM); DAD: 195 ± 10 nm with a reference of 360 ± 100 nm.

Preparation of Thiol Solutions

Preliminary Studies

Stability of Thiols

Solutions of CapSH, CySH, and GSH, were all prepared at concentrations of 6.5 x 10^{-4} mol dm^{-3} in deionised water. 1.00 mL of each thiol solution was transferred to 10.00 mL volumetric flasks containing Tris/HCl buffer solutions (pH 7.4; ionic strength (I) = 0.2 mol dm^{-3} (NaCl)) or HCl (pH 4.5; I = 0.2 mol dm^{-3} (NaCl)) and made up to the mark with deionised water for a final thiol concentration of 6.5 x 10^{-5} mol dm^{-3}.

A 1.0×10^{-4} mol dm^{-3} stock solution of ESSE was prepared in deionised water. 1.00 mL of each of the buffered thiol solutions and 1.00 mL of ESSE were mixed in 1 cm quartz cuvettes. The reactions were monitored spectrophotometically in the kinetic mode at 310 K at a cycle time of 10 s for 9000 s.

Variation of Acrylamide Concentration

1.00 mL of each thiol solution was transferred to 10.00 mL volumetric flasks containing Tris/HCl buffer solutions (pH 7.4; I = 0.2 mol dm^{-3} (NaCl)). A 1.0 mol dm^{-3} AA solution was prepared in deionised water and various amounts added to the buffer solutions for final AA concentrations ranging from 9.963×10^{-3} mol dm^{-3} to 9.963×10^{-2} mol dm^{-3} i.e. pseudo-first order conditions with respect to AA. 1.00 mL of each of the thiol buffer solutions and 1.00 mL of 1.0×10^{-4} mol dm^{-3} ESSE were added to quartz cuvettes at various times to measure the rate of thiol decomposition.

Variation of Thiol Concentration

Varying volumes of each thiol stock solution were added to 10.00 mL volumetric flasks containing Tris/HCl buffer solutions (pH 7.4; I = 0.2 mol dm^{-3} (NaCl)) to give final thiol concentrations within the range of 1.0×10^{-5} mol dm^{-3} to 1.0×10^{-4} mol dm^{-3}. The AA concentration was kept constant for each thiol. The AA concentration used for CapSH was 4.986×10^{-2} mol dm^{-3} and 9.963×10^{-3} mol dm^{-3} for both CySH and GSH. 1.00 mL of each of the thiol solutions and 1.00 mL of ESSE solution were added to quartz cuvettes at 30 s and 1 min intervals as described in **Preliminary Studies**. The reactions were monitored in the standard mode at a temperature at 310 K and the pseudo-first order rate constants, k_{obs}, for the thiol

decomposition were calculated.

Variable Temperature Studies

Values of k_{obs}, for each thiol were determined at three temperatures while varying the pH of the mixtures. The pH range used was 7.10 – 9.10 (Tris/HCl buffer). The ionic strength was kept constant at 0.2 mol dm^{-3} (NaCl). Thiol concentration was kept at 6.5 x 10^{-5} mol dm^{-3}, and the AA concentration was also kept constant for each thiol.

The AA concentration used for CapSH, CySH, and GSH were: 4.986 x 10^{-2} mol dm^{-3}, 9.963 x 10^{-3} mol dm^{-3}, and 1.993 x 10^{-2} mol dm^{-3}, respectively. GSH and CapSH solutions were monitored at 303, 310, and 315 K while CySH solutions were monitored at 293, 298, and 303 K.

Statistical Analysis of Data

The pseudo-first order rate constants, k_{obs}, for the thiol decomposition were calculated from absorbance time data using the StatGraphics Centurion XV.I Software (SGS). k_{obs} values at each temperature were used to calculate the specific rate constants, k, and the pK_s for each thiol. Activation parameters, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, $\Delta G^{\ddagger}$ for each thiol were calculated from Eyring plots. Parameters generated from the Eyring plots were used to construct an isokinetic plot.

Computational Studies

Gas phase (1 atm) geometries and energies were optimized using the BVP86 DFT functional [1] and Ahlrichs' TZVP basis set [2] as available in the PQS programme [3]. Transition state geometries were optimized until a single imaginary vibrational frequency corresponding to displacement along the intrinsic reaction coordinate was found. The energy differences between

reactants, transition states and products were computed relative to that of the combined energy of the isolated reactants in each case. Energy partition function analysis was used to determine the thermal corrections to the energy of each molecule (enthalpic and entropic) at different temperatures from translational, rotational and vibrational modes. With water as solvent, energies and optimized geometries were computed using the COSMO [4] electrostatic screening model and the output transferred to the ADF [5] programme for COSMO-RS [6] calculations at infinite dilution.

References

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