

## Towards a Deeper Understanding of the Mechanisms of Interaction between Acrylamide and Key Body-Fluid Thiols

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### Introduction

Acrylamide (AA), is an  $\alpha,\beta$ -unsaturated compound that is used mainly in the production of polyacrylamide. Polyacrylamide is non-toxic and is used as a flocculant in water treatment. However, the monomer AA is a known neurotoxin even with minimal exposure. It has also been classified as a possible human carcinogen. Human health effects associated with exposure to small amounts of AA over time are unknown [1-4].

AA was recently discovered in heat treated foods cooked above 100°C [5,6]. The Maillard reaction (the non-enzymatic browning reaction responsible for flavor in foods) is responsible for its formation in foods [7]. During heat treatment, naturally occurring amino acids, (namely asparagine) and reducing sugars, undergo a series of reactions resulting in the formation of AA in foods. AA content in food is directly related to the levels of these precursors. It is ironic, that foods which are deemed healthy such as: whole grain and wheat bran heat treated products contain higher concentrations of the toxin AA. This is because they naturally contain more asparagine and reducing sugars than their processed counterparts [8]. Additionally, methods which are deemed healthy such as: grilling, steaming, and roasting result in some of the highest levels of AA [9]. Products made with ammonium carbonate and bicarbonate may contain levels of AA up to several orders of magnitude higher than their sodium or potassium counterparts [9]. It is proposed that these ammonium salts act as either an additional source of nitrogen or catalyze the conversion of sugars to reactive carbonyls [10].

When ingested, AA may be converted to its more toxic metabolite glycidamide (GA), by enzyme-catalyzed reactions [7,11,12]. Both AA and GA are widely distributed in the body due to their high water solubility and small sizes [12]. They are also highly reactive with select biological compounds. AA and GA can undergo nucleophilic addition reactions via the C=C and the epoxide group, respectively [12]. The epoxide group of GA makes it more electrophilic than AA and can bind with DNA. GA-DNA adducts have been isolated in animal studies and is proposed to be the compound responsible for the genotoxicity of AA [13,14]. Studies have shown that AA will preferentially bind with proteins, free amino acids, and thiols, than with DNA [12,13].

It is proposed that detoxification of AA and GA occurs primarily by conjugation with glutathione (GSH) mediated by the enzyme glutathione-S-transferase and excreted in urine as mercapturic acid derivatives [7,11,13,15]. GSH is synthesized in the body from the thiol amino acids: L-cysteine and L-methionine [15,16]. Normal cell concentration of GSH is 5 mM [15]. Only AA and GA that cross the blood-brain barrier can cause toxic effects [17]. If GSH concentration is already low due to illness or malnutrition, then the possibility of AA-induced toxicity will be greater. This is especially relevant for school feeding programs in developing countries where nutritional support

for small children is often large scale, government sponsored and may include fortified baked goods.

Due to the toxic nature of AA and the fact that it is impossible to be eliminated from the diet, research is underway to minimize human exposure to AA. Methods currently being investigated include reducing the potential of AA formation by minimizing precursor content in foods before high-temperature cooking. Several pre-treatment methods have been published. For example, simple treatments on potato and potato products include: selecting cultivars that are known to contain low levels of precursors for high-temperature cooking and soaking samples in water before heat treatment as the precursors are water soluble [7,18,19]. More elaborate treatments include the addition of enzymes or microorganisms to minimize precursor content [7,19].

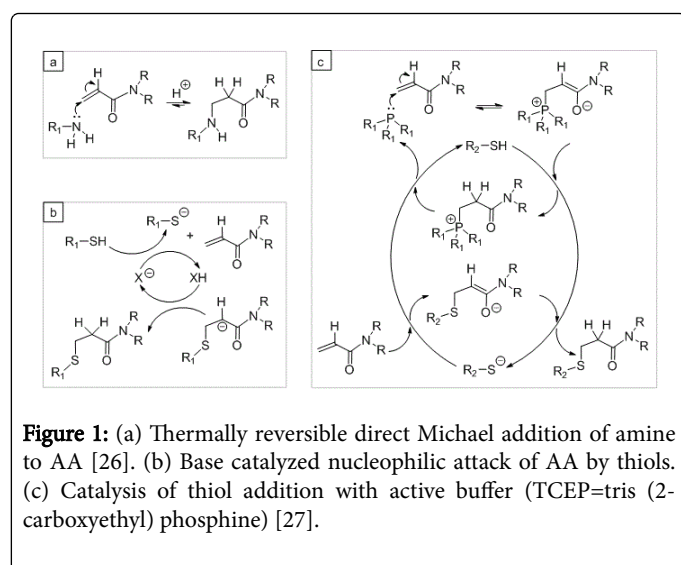
Alternatively, AA exposure may also be minimized by adding ingredients to food pre- or post- heat treatment which can react with the AA that would be formed thus resulting in an indirect reduction of available AA. Proposed post-heat treatment methods include: irradiation of the sample with UV light as AA becomes unstable after UV exposure, free radical polymerization of AA to the nontoxic polyacrylamide [7]. However, the feasibility of such methods may be difficult to implement in the food industry as they are expensive. Pre-heat treatment may include modification of the recipe to include compounds such as thiols and/or ascorbic acid [19]. These methods are cheaper than the previously mentioned post-heat treatment methods. However, modification of recipes may result in products which are aesthetically less appealing to consumers. These methods may result in a reduction in AA content but the formation of other toxic compounds. The health implications of such methods need to be further explored.

Scientists are currently attempting to understand AA's toxicity and potential carcinogenicity by exploring various mechanistic pathways and reactions both *in vivo* and *in vitro* [20]. It is generally accepted that AA (and similarly GA) react with amines and thiols via Michael-addition (Figure 1) [7]. As such, our group has investigated the reaction rates between AA and the thiols captopril (CapSH), L-cysteine (CySH), and GSH [17,21] which are commonly found in body-fluids. L-cysteine and GSH are naturally occurring thiols within the body. However, CapSH is man-made and is usually administered in the form of a drug used for the treatment of congestive heart failure. We expected that the rates of formation of the AA-thiol adducts would be directly in the order of: CySH > CapSH > GSH according to kinetic theory, given that this corresponds to the order of increasing molecular size of the thiol. However, our experimental observed rates were in the sequence: CySH > GSH > CapSH (Table 1). Given that the kinetic study isolated possible influences of pH, choice of buffer solution, concentrations of reactants and oxygen sensitivity in supporting

preliminary experiments, we sought mechanistic clues through ab-initio calculations using Density Functional Theory [22,23] for the molecules in the gas and solution (water) phase (Appendix).

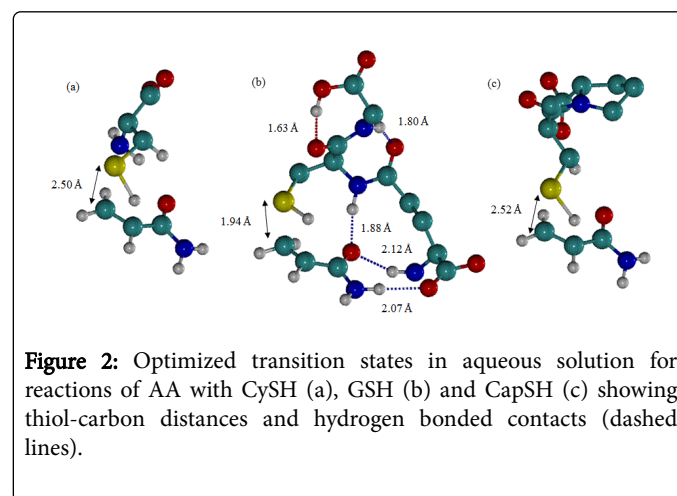
Parameter	CapSH	CySH	GSH
$k/\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$ (303 K)	$0.13 \pm 0.01$	$0.34 \pm 0.02$	$0.18 \pm 0.02$
Average $\text{pK}_s$	$9.01 \pm 0.05$	$7.4 \pm 0.2$	$8.5 \pm 0.2$
Literature $\text{pK}_s$ (298 K)	9.8 <sup>a</sup>	8.15 <sup>b</sup>	8.56 <sup>b</sup>
$\Delta H^\ddagger/\text{kJ mol}^{-1}$	$22 \pm 7$	$34 \pm 5$	$33 \pm 5$
$\Delta S^\ddagger/\text{J K}^{-1} \text{mol}^{-1}$	$-190 \pm 2$	$-143 \pm 2$	$-149 \pm 2$
$\Delta G^\ddagger$ (298 K)/ $\text{kJ mol}^{-1}$	$78 \pm 3$	$76 \pm 1$	$78 \pm 1$

**Table 1:** Calculated second-order rate constants (k), activation parameters, and  $\text{pK}_s$  for the reactions between AA, CapSH, CySH, and GSH at 303K. <sup>a</sup>ref [24]; <sup>b</sup>ref [25].



Comparative analysis of the computed transition states formed by the respective thiols provided evidence of close contact distances for allosteric hydrogen bonding interactions with GSH which are not available to CySH or CapSH (Figure 2). The developing carbon to thiol bond distance with GSH is also the shortest of the group by 22% in solvent. Together, these findings suggest that correlations of AA reactivity to acid dissociation constants or even computed nucleophilicity of the reactive (thiol or amide) group are likely to be insufficient for these biologically important compounds. Bayse [28,29] has employed a solvent assisted proton exchange (SAPE) method in DFT computations involving GSH, whereby several discrete water molecules bridge the reactants in a manner which couples nucleophilic attack by the thiol with a proton transfer through the bridge. We have been unable to improve upon our DFT results by including such additional discrete water molecules to date, probably because the strong intramolecular H-bonds in GSH are likely to be competitively superior to H-bonding in the bridging water molecules. In short, the water-free transition states are simply lower in energy than the water-bridged options. We intend to continue our theoretical investigations of these and related thiols (including the potentially more dangerous GA metabolite) with higher levels of theory so that the predicted

estimates of reaction rate constants and energetics will be further improved.



## Conclusion

Exposure to AA or its metabolite GA in foodstuffs, food residues, is a major concern today. Whether conjugation of AA or GA with GSH is the main route of reduction is a question of debate. Various amelioration techniques to remove/minimize precursor AA or GA contaminants using synthetic/natural enzymes or micro-organisms have been proposed. While thiol groups in amino acids are known to react via Michael-addition, whether thiol group is primary or secondary or both needs to be answered in order to determine the spectra of amino acids that are capable of reaction. Recent kinetic investigations done here with the intention of studying reactivity of various thiols; i.e. whether primary or secondary thiol affect reactivity. Furthermore, preliminary DFT calculations show that there is an open question of H-transfer via intramolecular GSH or H-transfer via water molecules. This debate needs further investigation.

## Appendix

### Computational studies

Gas phase (1 atm) geometries and energies were optimized using the BVP86 DFT functional [30] and Ahlrichs' TZVP basis set [31] as available in the PQS program [32]. 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 [33] electrostatic screening model and the output transferred to the ADF [34] program for COSMO-RS [35] calculations at infinite dilution.

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