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COMMUNICATION

In vitro Studies on the Reaction Rates of Acrylamide with the Key Body-fluid Thiols: *L*-Cysteine, Glutathione, and Captopril

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The mechanisms of interaction between acrylamide and *L*-cysteine, glutathione and captopril were studied *in vitro*. Experimental second order rate constants calculated at 303 K were: 0.34 ± 0.02, 0.18 ± 0.02, and 0.13 ± 0.01 dm^3 mol^{-1} s^{-1} for *L*-cysteine, glutathione, and captopril, respectively, potentially involving inter- and intra-molecular H-bonding in the acrylamide-glutathione complex.

Keywords: acrylamide, *L*-cysteine, glutathione, captopril, *in vitro* studies, reaction kinetics

Introduction

Acrylamide (AA) is a known neurotoxin and a potential human carcinogen [1]. Chromatographic studies have confirmed that AA is formed in foods cooked above 100 °C (373 K) as a result of the Maillard reaction [2]. This implies that cooking methods such as: steaming, baking, and roasting, which are deemed healthy, may result in the formation of AA in foods [3].

When ingested, AA may be converted to glycidamide (GA) by the enzyme cytochrome P450-2E1 [4]. The formed GA is much more reactive than AA and can mutate DNA by conjugation with the amine groups of purine bases [5-7].

Both formed AA and GA are detoxified by glutathione conjugation mediated by the enzyme glutathione-S-transferase and their metabolites are excreted in urine, which constitutes one of the major metabolism/detoxification routes of AA [4, 8].

α,β-unsaturated carbonyl compounds like AA react readily with thiolate (RS^-) anions and amine groups by Michael-addition reactions [4]. However, the relationship of such reactions to AA's toxicity and potential carcinogenicity is unknown.

Since AA can have deleterious effects on the human body, and may be ingested in large amounts in the daily diet [3]; we embarked on a study to understand the mechanisms of interaction between AA and thiols (RSH). Here, we comprehensively report on *in vitro* studies involving the reactions of AA with the following key body-fluid thiols: *L*-cysteine (CySH), glutathione (GSH) and captopril (CapSH). This is the first report on the reactions rates of AA with the thiol CapSH with Density Functional Theory (DFT) computations.

GSH is the most abundant thiol in the human body; present at cellular concentration of 5 mM in non-smokers. GSH acts as an alternative nucleophile instead of nucleophilic portions of proteins and/or DNA [9] and thereby providing protection against toxic electrophiles such as free radicals and AA within the cell [10].

CySH is synthesised in the body, if sufficient methionine is available, and is a precursor for the biosynthesis of GSH. GSH taken orally does not absorb well across the gastrointestinal tract [10, 11]. CySH residues are essential in maintaining the structure of proteins and enzymes such as insulin and cytochrome P450.

The thiol CapSH is prescribed for the treatment of hypertension and congestive heart failure [12]. It acts as an angiotensin converting enzyme (ACE) inhibitor and thus reduces the formation of angiotensin I from angiotension II; angiotensin I causes a constriction of the blood vessels and hence an increase in blood pressure. CapSH is also known to inhibit the production of superoxides and scavenge free radicals [13].

How AA reacts with these thiols, thus reducing or enhancing their ability to carry out their function within the body, is of great importance in understanding the mechanism and metabolism of AA.

Results and Discussion

Formation of the AA–SR adducts was confirmed by HPLC-MS analysis, thus supporting the Michael-addition reaction mechanism. Assuming that the nucleophilicity of all three thiols is approximately the same, the rates of the reaction with AA were expected to follow: CySH > CapSH > GSH, reflecting the increasing molecular size of the thiols. The Gibbs free energies of activation for the solvated thiols calculated at 303.15 K with DFT are 52.17, 50.90, and 60.56 kcal mol^{-1} for CySH, CapSH, and GSH, respectively, implying that GSH should have the slowest reaction rate due to the high activation energy barrier. However, the experimental second order rate constants at 303 K were 0.34 ± 0.02, 0.13 ± 0.01 and 0.18 ± 0.02dm^3 mol^{-1} s^{-1} for CySH, CapSH, and GSH, respectively, with apparent order CySH > GSH > CapSH.

We briefly propose here that although GSH is a much larger molecule and is less diffusive than CapSH, the transition state with GSH is stabilized by intra- and inter-molecular hydrogen bonding while such interactions are absent in the CapSH adduct. A detailed theoretical study of the effects of hydrogen bonding and solvation energetics on the activation energy for the reactions of AA with the subject thiols will be presented as a full paper.

Greater exposure to AA whether orally or dermally, will deplete the body's thiol concentration and hence increase AA toxicity. This can be further exasperated by malnutrition caused

by diets lacking in sulphur-containing amino acids; especially CySH and methionine which are essential for GSH formation. GSH levels may also be reduced by defects caused by liver damage due to alcoholic hepatitis, and cirrhosis [4].

Only AA that crosses the thiol barrier is available to cause neurotoxicity and be enzymatically converted to GA. *In vitro* studies conducted with *N*-acetyl-*L*-cysteine and GSH prevented the alkylation of DNA by AA. Vitamin B_6, and sodium pyruvate have been shown to prevent or reduce AA-induced neuropathy [4].

It is possible to reduce AA formation in foods but impossible to eliminate AA from the diet. Once ingested, AA will undergo conjugation reactions within the body. Since the reaction between AA and certain thiols such as CySH is fast, the potential of its addition to foods before or after preparation to reduce the bio-availability of AA and enhance food safety could in turn prevent adverse cellular effects of AA and GA [8]. Such preventative measures need to be tested for their feasibility.

Our studies show that rate of reaction between AA and GSH is faster than that between AA and CapSH; as such AA will preferentially bind with GSH than CapSH. This implies that detoxification of AA would proceed primarily via conjugation with GSH. However, if GSH concentration is low then a competitive reaction could exist between nitric oxide [14] and AA for the thiol group of CapSH. This could have serious health implications for hypertensive patients as the effects of the drug would be compromised.

Conclusions

Kinetic studies, HPLC-MS analysis and DFT computations for the reaction between AA and CySH, CapSH and GSH support a Michael-addition reaction mechanism which may account for the observed carcinogenicity and biological toxicity of AA *in vivo*. CySH showed the fastest reaction with AA and could therefore be added to high-temperature-prepared foods as both a nutritional supplement and also to alleviate the potential of AA toxicity. We expected the reaction between CapSH and AA to be faster than that of GSH due to the larger molecular size of the latter, but observed a reverse order instead. Assuming similar nucleophilicity of the thiol groups, we propose from molecular modelling that the transition state of the AA–SG adduct is stabilized by intra- and inter-molecular hydrogen bonding while such interactions are absent in the AA–SCap adduct, accounting for the observed faster reaction with GSH.

Notes

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References

1. I.A.R.C., I.A.R.C. Monogr. Eval. Carcinog. Risks Hum., 1994, 60, 389 - 433.
2. E. Tareke, P. Rydberg, P. Karlsson, M. Törnqvist and S. Eriksson, Chem. Res. Toxicol., 2000, 13, 517 - 522.
3. G.-A. Bent, P. Maragh and T. Dasgupta, Food Chem. , 2012, 133, 451-457.
4. M. Friedman, J. Agric. Food Chem., 2003, 51, 4504 - 4526.
5. D. Deorge, G. Gamboa de Costa, M. Churchwell, L. Von Tungeln, P. Hamilton, F. Beland and M. Marques, Chem. Res. Toxicol., 2003, 16, 1328-1337.
6. R. Lopachin and A. DeCaprio, Toxicol. Sci, 2005, 86, 214 - 225.
7. K. Galesa, U. Bren, A. Kranjc and J. Mavri, J. Agric. Food Chem., 2008, 56, 8720-8727.
8. H. Kurebayashi and Y. Ohno, Arch. Toxicol., 2006, 80, 820 - 828.
9. K. Kolšek, M. Sollner Dolenc and J. Mavri, Chem. Res. Toxic., 2013, 26, 106-111.
10. J. Castell, M. Gomez-Lechon, X. Ponsoda and R. Bort, Arch. Toxicol., 1997, 313 - 321.
11. Resolver2009, HubPages, vol. 2014.
12. P. Ribeiro, A. Santini, H. Pezza and L. Pezza, Eclet. Quim., 2010, 35, 179-188.
13. O. Pecháňova, Physiol. Res., 2007, 56 S41-S48.
14. R. M. Clancy, D. Levartovsky, J. Leszczynska-Piziak, J. Yegudin and S. B. Abramson, Proc. Natl. Acad. Sci. USA, 1994, 91, 3680-3684.