

PAPER



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Kinetics and mechanism of amino acid derived 2-thiohydantoin formation reactions†

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The reaction of allyl isothiocyanate with some common natural protein amino acids (glycine, L-alanine, L-valine, L-leucine and L-phenylalanine) was monitored. ¹H NMR spectroscopy is used as a practical tool in searching for the intermediates and determining the kinetic parameters of the reaction. The density functional theory (DFT) method is utilized to better apprehend the mechanisms of thiohydantoin formation and kinetics through which these processes are facilitated, as well as for studying important intermediates and transition states. Based on our findings, a two-step mechanism in which the key reaction step is cyclization *via* an internal nucleophile in the doubly charged intermediate product is proposed. The theoretical calculations are in good agreement with our experimental observations, and the reactivity of the amino acid esters is compared and discussed.

Introduction

Hydantoins represent a large family of five-membered cyclic ureides with many intriguing physical and chemical properties.¹ They encompass a wide range of structurally diverse and synthetically attractive compounds.² Hydantoins exhibit many different biological and agricultural activities, and many of them are frequently used in medicine and therapy as marketed drugs.^{2,3} Their sulfur analogues, 2-thiohydantoins, belong to an important subgroup of the hydantoin family due to their activities, such as anticancer,^{4,5} antimicrobial,^{6,7} insecticidal and fungicidal,^{8–10} antimutagenic,¹¹ antithyroidal,¹² anti-ulcer and anti-inflammatory¹³ and anticonvulsant activities.¹⁴ 2-Thiohydantoins are also known for their use as reference standards in C-terminal protein sequencing¹⁵ and pesticides,¹⁶ as well as reagents for dye development.¹⁷

There are many synthetic routes that lead to the formation of thiohydantoins,¹⁸ but one of the more important and frequently used ones is the synthesis from amino acids. The formation of amino acid-derived 2-thiohydantoins does not require rigorous chemical conditions, and thus, it is plausible for them to form under physiological conditions.¹¹

2-Thiohydantoins can be synthesized from amino acids in a number of ways, most notably in the reaction with alkyl or aryl isothiocyanates.¹⁸ Allyl isothiocyanate (AITC) is the main pungent constituent of horse-radish and black mustard and

is a staple in the food industry, often used as a food additive. It readily reacts with free amino acids or amino acids in protein chains, yielding 2-thiohydantoin derivatives.¹¹ 2-Thiohydantoins can possibly form during food cooking processes or during the metabolism of ingested compounds. This, of course, raises a question about the chemical and biological implications of the presence of these amino acid-derived 2-thiohydantoins and, accordingly, the elucidation of the important mechanisms of their formation gains.

One of the pioneering insights into the mechanisms of thiohydantoin formation is certainly Edman's work on peptide sequencing.¹⁹ This method, called 'Edman degradation', involves a one-by-one reaction of amino acids at the N-terminus with phenyl isothiocyanate, which produces the corresponding 2-thiohydantoins. Even though the methodology has received some critics and addendums,^{20,21} it is still an important landmark for not only protein sequencing but also hydantoin chemistry as a whole.

To the best of our knowledge, a kinetic study that explores the reactions of amino acid derived 2-thiohydantoin formation has not been done yet. With all things taken into account, we propose that a mechanistic, kinetic and theoretical study such as this one would prove beneficial for better understanding of the mechanisms of thiohydantoin formation.

Experimental

General

All chemicals and reagents are commercially available and were used as received without further purification. Solvents were purified by distillation prior to use. Anhydrous methanol was prepared by a standard drying procedure.

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