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Reaction profiling of a set of acrylamide-based human tissue transglutaminase inhibitors

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ABSTRACT

The major function of the enzyme human tissue transglutaminase (TG2) is the crosslinking of proteins via a transamidation between the γ-carboxamide of a glutamine and the ε-amino group of a lysine. Overexpression of TG2 can lead to undesirable outcomes and has been linked to conditions such as fibrosis, celiac disease and neurodegenerative diseases. Accordingly, TG2 is a tempting drug target. The most effective TG2 inhibitors to date are small-molecule peptidomimetics featuring electrophilic warheads that irreversibly modify the active site catalytic cysteine (CYS277). In an effort to facilitate the design of such TG2 inhibitors, we undertook a quantum mechanical reaction profiling of the Michael reaction between a set of six acrylamide-based known TG2 inhibitors and the TG2 CYS277. The inhibitors were docked into the active site and the coordinates were refined by MD simulations prior to modelling the covalent modification of the CYS277 thiolate. The results of QM/MM MD umbrella sampling applied to reaction coordinates driving the Michael reaction are presented for two approximations of the Michael reaction: a concerted reaction (simultaneous thiolate attack onto the acrylamide warhead and pronation from the adjacent HIS335) and a two-stage reaction (consecutive thiolate attack and protonation). The two-stage approximation of the Michael reaction gave the better results for the evaluation of acrylamide-based potential TG2 inhibitors *in silico*. Good correlations were observed between the experimental TG2 IC_{50} data and the calculated activation energies over the range 0.0061–6.3 μM (three orders of magnitude) and we propose that this approach may be used to evaluate acrylamide-based potential TG2 inhibitors.

1. Introduction

Human tissue transglutaminase, also known as transglutaminase 2 (TG2) is a member of the transglutaminase family of enzymes. A major function of TG2 is the crosslinking of proteins via a calcium-dependent transamidation between the γ-carboxamide of a glutamine and an ε-amino group of a lysine [1]. Physiologically, TG2 activity is associated with regulation of the extracellular matrix (ECM) formation, cell adhesion, wound healing, signal transduction, apoptosis, and stabilisation of skin and hair [1–3].

Overexpression of TG2, however, can lead to undesirable outcomes. For example, increased TG2 activity has been noted in neurodegenerative diseases such as Parkinson's, Alzheimer's and Huntington's [4–6] as well as in celiac disease [7]. Furthermore, the inhibition of TG2 activity has been shown to reduce metastasis in pancreatic and lung cancers [8,9]. Three of the major classes of TG inhibitors that have been exemplified include: competitive reversible inhibitors such as cystamine [10,11]; reversible non-competitive inhibitors that act by binding to an allosteric site within TG2 preventing its activation [12,13]; irreversible inhibitors containing an electrophilic warhead that act by alkylating the key active site cysteine. (References given below) In the biological setting, any inhibitor targeting the active site of TG2 has to overcome the locally high total concentration of potential substrates, for example competition with fibronectin vitronectin, osteonectin, osteopontin, laminin, fibrillin, nidogen, collagens I, II V and XI in the extracellular matrix [14]. For this reason, despite reservations over potential toxicity and off-target activity, irreversible inhibitors may, in the end, provide therapeutic inhibitors of this enzyme. Most of the irreversible inhibitors published to date are peptidic or peptidomimetic and contain an electrophilic group capable of reacting with a cysteine thiolate anion, for example, maleimides, epoxides, acrylamides, chloroacetamides, beta-keto sulfonium ions and substituted imadazolium groups [15–21].

Following on from a series of irreversible dipeptide-based inhibitors (Fig. 1**a**) featuring the beta-keto sulfonium warhead [16],

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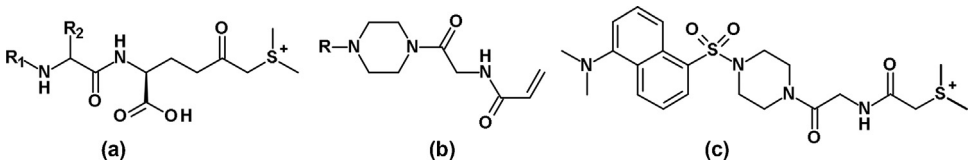

Fig. 1. Irreversible TG2 inhibitor types and a fluorescent example published by the Griffin group.

the Griffin group has published a set of conformationally-restricted inhibitors (Fig. 1**b**) featuring the piperazinyl-glycylacrylamide core structure [15,21].

The piperazine portion serves to constrain the flexibility of the central region of the compounds and the glycylacrylamide is able to reach into the active site tunnel to allow a Michael reaction to occur between the thiolate anion of CYS277 and the beta carbon of the acrylamide. The other piperazine nitrogen is bound to a series of hydrophobic entities via amide, carbamate and sulfonamide linkages. In support of the presumed site of reaction for the acrylamide compounds being CYS277, the Griffin group [15] has profiled a close analogue of acrylamide compound **5** (Table 1) featuring a beta-keto sulfonium warhead in place of the acrylamide (Fig. 1**(c)**). Recombinant wild-type TG2 and its active site mutant CYS277SER were incubated with the compound. Subsequently, TG2 was separated on denaturing SDSPAGE and western blotted using an anti-dansyl antibody. The observed bands indicated that the inhibitor had been covalently incorporated into the wild-type enzyme but not the CYS277SER mutant form.

Our initial attempts to use computer-based protein-ligand docking methods (data not presented) to search for irreversible inhibitors of TG2 were successful to the extent of distinguishing between moderate-high potency inhibitors and non-inhibitor analogues. It was not possible, however, to rank compounds according to potency at the good-high potency end. In retrospect this is not surprising, since in the case of irreversible covalent binding of the inhibitor to the active site, placement of the inhibitor in the active site is only one part of the overall process. For example, it is possible for a compound to fit very well into the active site but for the subtleties of its arrangement in the active site to impair its ability to derivatise the CYS277 thiolate anion. Therefore, as an attempt to develop a computational screening tool to allow the ranking of acrylamide-containing small molecules as irreversible TG2 inhibitors, we decided to implement a QM/MM umbrella sampling approach to investigate the covalent bond-forming stage. Thus the overall approach was to dock the potential inhibitors into the active site of a TG2 model, conduct MD upon the complexes, select plausible starting positions for an approximation to the thiolate-acrylamide Michael reaction and to probe the energy profile of the reaction coordinate via umbrella sampling.

2. Methods

MM-MD simulations were performed using a CUDA-ported version of Amber 12 [22–25] on workstations and servers equipped with Nvidia GTX 780, GTX 780 Ti or Titan graphics cards. QM MM-MD hybrid calculations [26,27] were performed using Amber 12 on an Intel Xeon-based server. The trajectories were processed using PTRAJ or CPPTRAJ [28]. The production phases of all MD trajectories were analysed with respect to temperature, pressure and RMSD and found to be stable.

2.1. The inhibitors

Six irreversible TG2 inhibitors featuring an acrylamide warhead were used in this work (Table 1). They were adopted from Badarau et al [15].

2.2. Preparation of TG2 active site models and complexes

Our models for the open form of TG2 were derived from a repaired version of the Protein Data Bank entry 2Q3Z [29] in which the missing residues were added and the existing covalently-bound inhibitor was deleted. CYS277 was set to its ionised form and HIS335 was allocated its protonated form following the generally accepted view that the catalytic triad CYS277, HIS335, ASP358 is arranged such that the cysteine and the histidine exist as a thiolate-imidazolium pair stabilised by hydrogen bonding to ASP358 [30,31]. In order to maximise the computational speed, the sandwich and barrel domains at the furthest extremes of the protein structure (residues 1–154 and 586–683) were deleted. During the course of an exploratory 250 ns MD simulation of the entire enzyme in explicit water, the amino acid residues within 8 Å of CYS277 were found to have an average RMSD of 0.82 Å using the first frame as a reference. Furthermore, the catalytic triad residues CYS277, HIS335, ASP358 exhibited individual RMSF values of 0.38, 0.31 and 0.36 Å respectively. The same procedure applied to the truncated enzyme resulted in an average RMSD of 0.92 Å and RMSF values for CYS277, HIS335 and ASP358 of 0.33, 0.30 and 0.35 Å respectively. This supported our view that the peripheral sandwich and barrel domains were too far away from the active site core region to have any effect upon the active site in the timescale of the simulations reported in this work. These regions are identified in yellow in Fig. S**1a** in the supplementary information.

The truncated version of the repaired enzyme was relaxed using a combination of energy minimisation and molecular dynamics (100 ns production phase) in explicit water using the Amber 12 package. Further details are given in the supplementary information. Models for the active site were taken from the resultant trajectory and probed by docking active TG2 irreversible inhibitors taken from the work of Badarau et al. [14] and Griffin et al. [16] using the programs CACHe WorkSystem Pro (version 7.5.0.85, Fujitsu Limited, Tokyo, Japan 2006) and GOLD Suite (version 5.2.2, CCDC Software Limited, Cambridge, UK 2013) (flexible ligand/flexible active site residue side chains). Six appropriate docking complexes were selected on the basis of two criteria: a maximum distance of 4 Å between the inhibitors' warhead electrophilic carbon and the CYS277 thiolate; the hydrophobic portion of the inhibitors being satisfactorily buried under the helix 309 – 318 as seen in the crystal structure 2Q3Z.pdb. The complexes were subjected to unrestrained MD in explicit water for 275 ns from which 6 models of TG2 active site were generated. The acrylamide-based inhibitors (**1**–**6**, Table 1) were docked into the resulting active site models. Complexes for these inhibitors were selected using the criteria given above and these were subjected to a further 5 ns of molecular dynamics simulation at 300 K in explicit water.

The complexes that were carried through to the umbrella sampling were either docked complexes from the penultimate stage above (**3**, **4** and **6**) or were snapshots from the final 5 ns MD simulations (**1**, **2** and **5**). For each compound, the structure selected was the one that had the lowest distance between the thiolate sulfur of CYS277 and the compound's electrophilic carbon. Good docking rank and favourable interactions with active site residues (for example, hydrogen bonding with GLN276, ASN333 and PHE334) were also considered in the selection process.