Bioorganic Chemistry 38 (2010) 234–241

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

The binding site of zinc and indium metal to amino acid derivatized squarate complexes – Implications in inhibitor and mediator designs

Natasha Ramroop-Singh [a,*], Dyer Narinesingh [a], Gurdial Singh [a], Christopher T. Seto [b] Anthony B. Comeau [b]

[a] Department of Chemistry, The University of The West Indies, St. Augustine, Trinidad and Tobago
[b] Department of Chemistry, Brown University, Providence, Rhode Island 02912, USA

ARTICLE INFO

Article history:
Received 29 April 2010
Available online 15 June 2010

Keywords:
Squaric acid
Metalloprotease inhibitors
Glucose biosensor mediators
Ionic liquids

ABSTRACT

Three novel metal squaric acid–peptide complexes, SQI–SQIII were prepared by addition of indium triflate or zinc chloride to the previously reported compounds [1], 3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione (squarate 1), and 3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one (squarate 2). The structures of SQI–SQIII were elucidated using NMR analysis. The electrochemical applications of two of these metal–squaric acid systems (SQI and SQII) were also investigated. Incorporation of SQII as a mediator, in the previously optimized Pt/p(HEMA)/p(pyrrole)/GOx electrode using the ionic liquid [bmim][BF_4] as the solvent medium, produced a biosensor with enhanced properties, namely a sensitivity of 175.9 mA/M D-glucose, working potential of +200 mV, large linear range (0–12 mM) and a detection limit of 1×10^{-6} M.

1. Introduction

Matrix metalloproteases (MMPs) are zinc dependant enzymes that function at neutral pH and are intimately involved in the remodeling of the extracellular matrix. This family of endopeptidases enzymes plays a pivotal role in the regulation and composition of the matrix resulting in the maintenance of the normal physiology of the tissues. In particular, they have an essential role in a number of processes including development of embryos, healing and reproduction. As a result of this broad spectrum of activity, these enzymes have become attractive targets for the synthesis of therapeutic inhibitors for the potential treatment of inflammation, degenerative and malignant diseases. The synthesis and properties of the MMPs have been reviewed extensively during the past 15 years [1].

One of the main therapeutic focus of these inhibitors has been directed at preventing metastatic growth and related angiogenesis where MMPs are considered crucial, as well as in the treatment of rheumatism and central nervous system diseases [2], and for applications in oncology [3], and cancer therapy [4]. Development of MMP inhibitors has been based on known interactions between the enzyme and their substrates/inhibitors in order to design molecules that specifically chelate the zinc ion and block the active site. These new inhibitors can be divided into carboxylates and amino-carboxylates, phosphinates, sulphydryl derivatives, and hydroxamates, of which the hydroxamates are considered to possess the most potent zinc binding group [5].

As part of ongoing efforts targeted at finding new inhibitors for MMPs, novel peptidothiosquarate compounds that exhibit IC_{50} values of 15 μM against bMMP-1 have been prepared.[6] In these reports by Onaran et al. [6] the metal ions were proposed to bind at the expected sites on the squarates (Fig. 1a and b). As a result of these findings, we sought to elucidate the binding site(s) of indium and zinc ions to the amino acid derivatized squarate conjugate inhibitors, 3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione (Fig. 2a) and 3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one (Fig. 2b). In addition to the binding site reported by Onaran et al. [6] evidence is given in this report for another site for complexation of the metal ions to the squarate complexes (Fig. 1c), but this data produced does not conclusively rule out the previously proposed binding mode.

Also investigated is the potential of two of these complexes to act as mediators, to significantly lower the working potential of a previously optimized glucose biosensor. The synthesis and characterization of various metal squarate complexes have been reported to date. These include Cu, Fe, Zn, Al, Ni, Mn, Co, Ca, Mg, Mo, Gd, La, Eu and Tb [7] metal complexes. Also the binding mode of metals to squaric acid systems and homologous structures such as hydroxamic acid has been previously postulated [8] with the binding/complexation site between the N–OH group and the ketonic function of the squarates. The site at which the metal of the enzyme binds to the inhibitor has obvious implications with respect to the design and structure of the inhibitor. The binding mode of metals to

* Corresponding author.
E-mail address: natasha.ramroopsingh@utt.edu.tt (N. Ramroop-Singh).

0045-2068/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2010.06.001

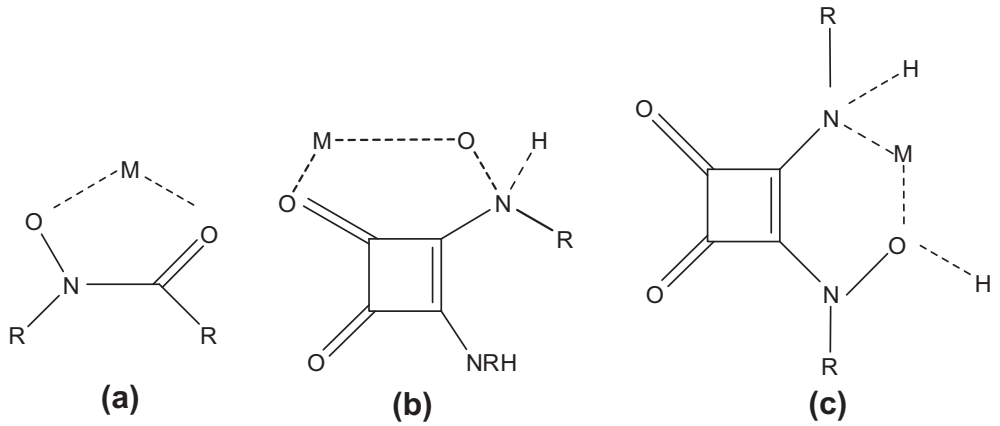

Fig. 1. (a) Known binding mode of hydroxamic acids to metal ions [9]. (b) Originally proposed binding mode of squaric acids to metal ions [6]. (c) New proposed binding mode of squaric acids to metal ions.

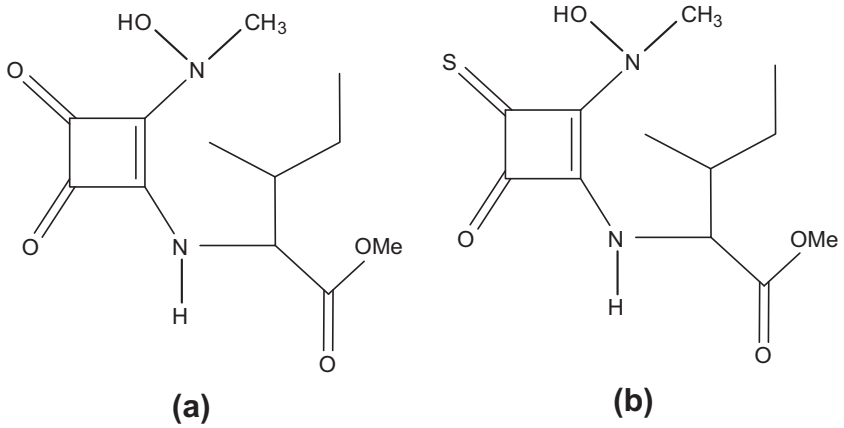

Fig. 2. (a) 3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione (squarate 1). (b) 3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one (squarate 2).

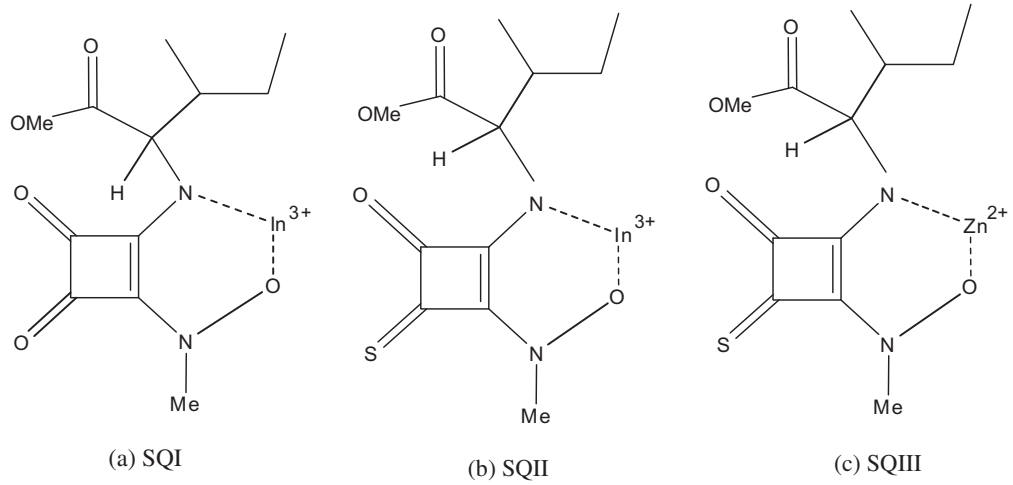

Fig. 3. (a) SQI synthesized by complexation of squarate 1 with indium triflate. (b) SQII synthesized by complexation of squarate 2 with indium triflate. (c) SQIII synthesized by complexation of squarate 2 with zinc chloride.

hydroxamic acids (Fig. 1a) have been elucidated and confirmed [9]. Squaric acid and its derivatives, since possessing an analogous structure to hydroxamic acid, have been assumed to bear a similar if not identical binding mode to metals (Fig. 1b).

We wished to gain an understanding of the binding of zinc ions to these peptide squarates, as zinc is the bioactive metal present in matrix metalloproteases. In addition, we also investigated the incorporation of indium ions into these systems as its excellent