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

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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

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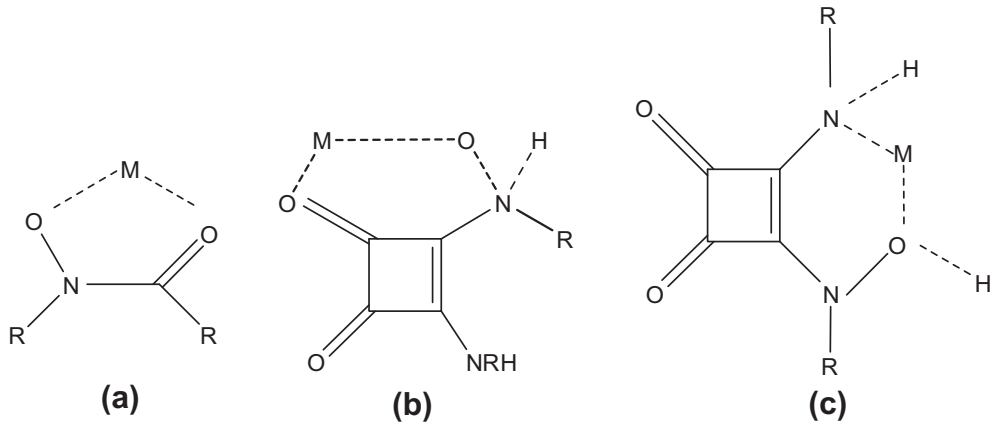


Fig. 1. (a) Known binding mode of hydroxamic acids to metal ions [9]. (b) Originally proposed binding mode of squaric acids to metal ions [6]. (c) New proposed binding mode of squaric acids to metal ions.

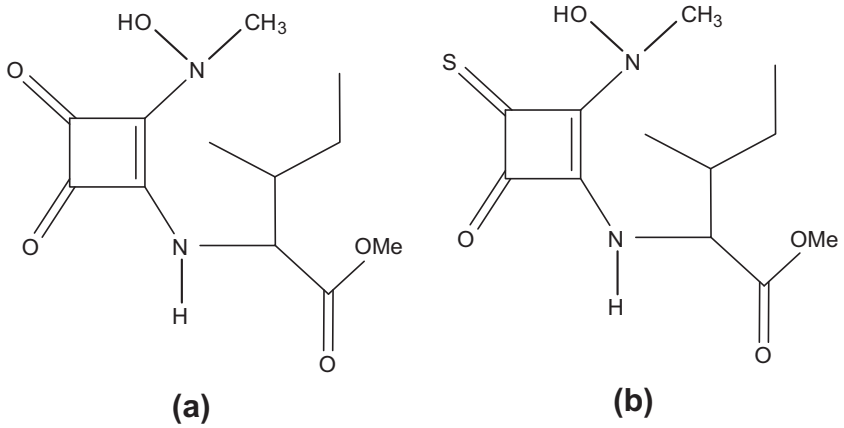


Fig. 2. (a) 3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione (squarate 1). (b) 3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one (squarate 2).

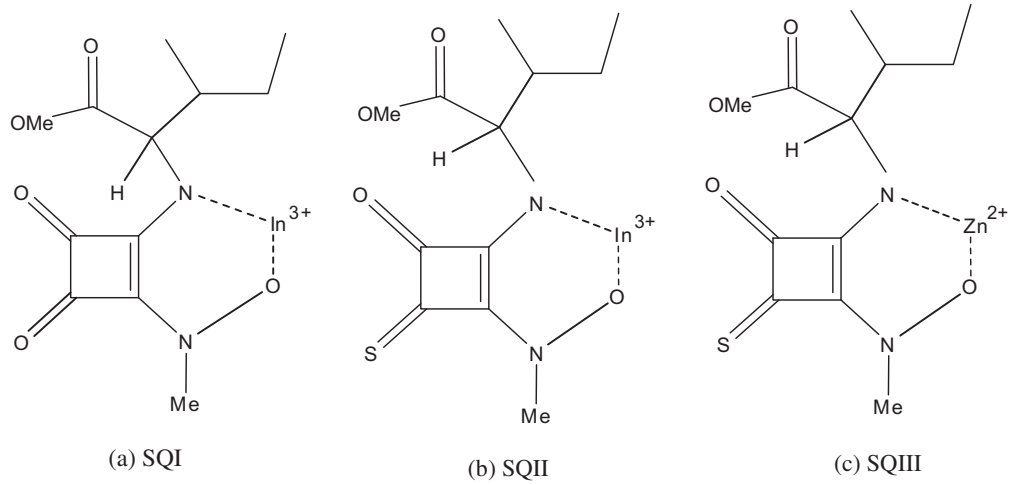


Fig. 3. (a) SQI synthesized by complexation of squarate 1 with indium triflate. (b) SQII synthesized by complexation of squarate 2 with indium triflate. (c) SQIII synthesized by complexation of squarate 2 with zinc chloride.

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

conductivity and relative inherent stability makes it an ideal metal for conducting complexation and electrochemical investigations. Furthermore, indium is able to change its oxidation states readily and the differences in the oxidation potentials of +3 to +1 are among the lowest of the elements. The nuclear spin of In-115, measured by Marino and co-workers was found to be $I = 9/2$ [10] and indium oxide has been used to prepare electrodes for monitoring physiological responses in cells [11], as well as in the production of nanowire material devices for biosensing applications [12].

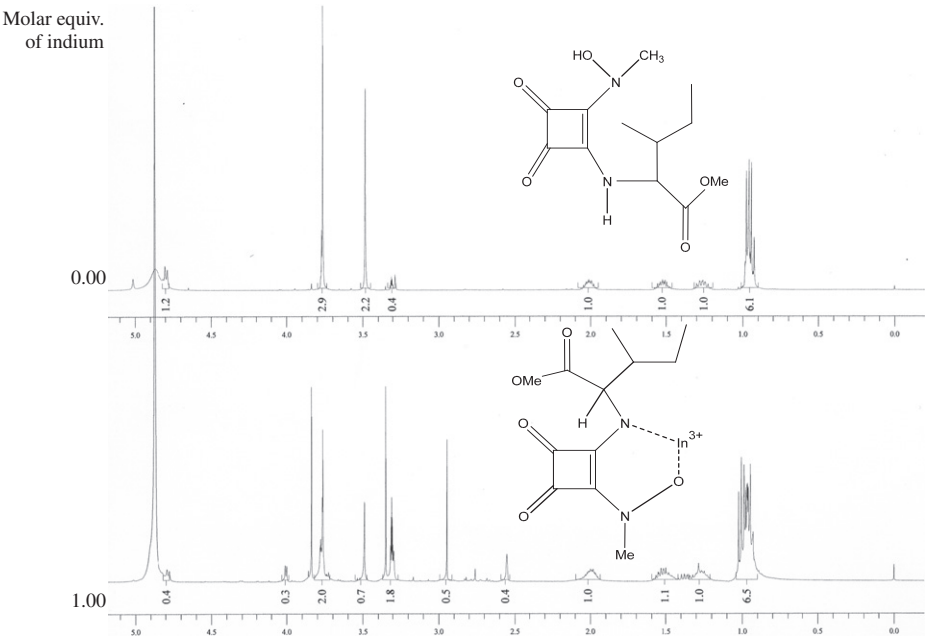
2. Results and discussion

Upon addition of an equimolar amount of indium triflate to squarate 1 in d^4-MeOH, (Scan 1), there is a splitting of the N-methyl protons, from a δ of 3.482 ppm (s, 3H) to chemical shifts of 3.487 (s, 1H), 3.309 (m, 1H) and 2.947 (s, 1H), while the α proton of the Ile side chain (chiral proton) undergoes a shift from 4.801 ppm (s, 1H) to 4.012 ppm. All other signals remain approximately constant. The ^{13}C NMR spectra of SQI indicate the triflate carbons in the region of 115–126 ppm, and no signals in this region were seen in the ^{13}C NMR of squarate 1. This evidence indicates a complexation of the indium metal to the two nitrogen atoms of the side chains of the squarate (Fig. 1c), and hence deviates from the typically proposed binding site, which is a binding to the hydroxyl group on the N-methyl substituent and the oxygen double bond of the squarate (Fig. 1b). Upon increasing additions of indium triflate (from 0 to 1 equimolar amounts) to squarate 2 in $CDCl_3$ (Scan 2), to produce SQII, there is again, a dramatic shift in the δ value of the chiral proton of squarate 2 from 5.867 (br s, 1H) to 5.009 (s, 1H) in SQII, while the β proton of the Ile side chain also gradually shifts from 2.056 ppm (m, 1H) to 2.168 ppm (m, 1H). All other proton signals remain constant. This again, indicates complexation of the metal to the two nitrogen atoms of the side chains of the squarate, as seen with SQI. There is a negligible change in the N-methyl protons, on looking only at the initial and final spectra of Scan 2 (which was observed with SQI in d^4-MeOH) but the fact that the complex is now in a different solvent system must be taken into consideration. Also, it must be noted (Scan 2) that from the ^1H NMR scan of exactly 0.5 M equivalent of indium triflate present with squarate 2, which can be considered the mid-point stage between the free squarate and the complexed form, the N-methyl proton signals almost disappear, indicating some sort of participation in the complexation process. There is a gradual disappearance of the protons of the –NH and –OH groups (as they are exchanged) due to the presence of a small but increasing amount of deuterated methanol in the system, as the indium triflate does not completely dissolve in $CDCl_3$ and some CD_3OD had to have been added. Also, due to the hygroscopic nature of indium triflate, there is the gradual appearance of a water peak at a δ of approximately 1.2–1.3 ppm (Scan 2).

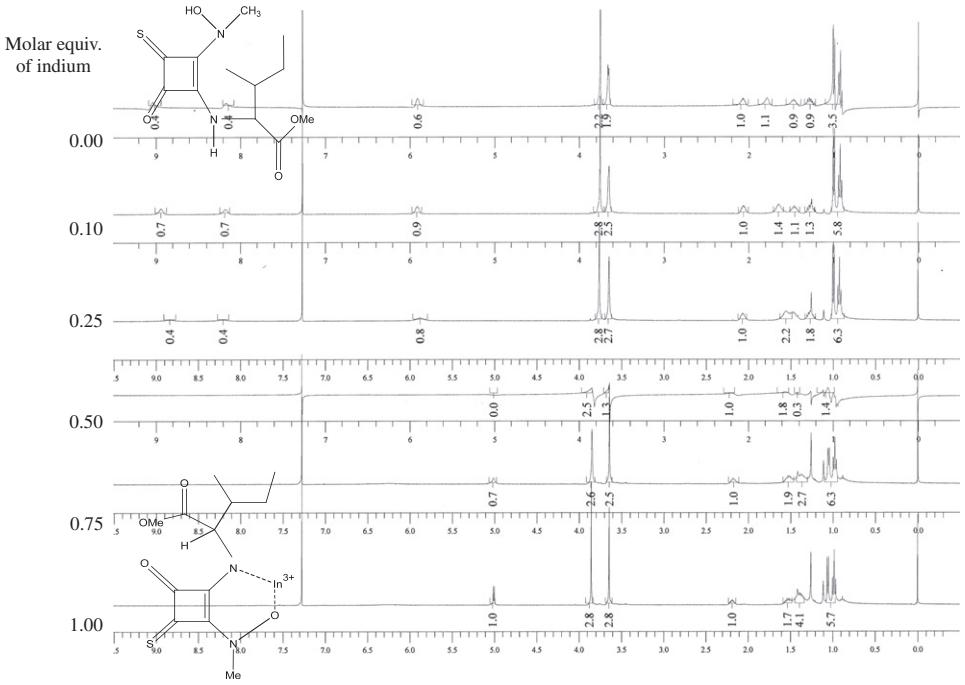
A repetition of the increasing additions of indium triflate to squarate 2, this time using d^4-MeOH as the solvent (Scan 3), indicates again that complexation of the metal occurs between the two nitrogen atoms of the side chains present on the squarate. There is a similar splitting of the N-methyl protons as with SQI from 3.607 (s, 3H) in squarate 2 to 3.585 (s, 1H), 3.492 (s, 1H) and 3.325 (m, 1H) in SQII, as well as a shift of the chiral proton from 5.997 ppm (s, 1H) to 4.008 ppm (s, 1H).

The design of a glucose biosensor incorporating SQI into the gel formulation of the electrode demonstrates the superiority of the newly formed conducting band. Firstly, four electrodes were evaluated for their response to a fixed concentration of glucose (Fig. 4). No appreciable current values were generated in the presence of p(pyrrole), p(pyrrole) and squarate 1, or p(pyrrole) and indium triflate only (electrodes 1–3). However, the combination of indium triflate and squarate 1 (effectively SQI) in the p(pyrrole) gel matrix (electrode 4) causes an appreciable current at about +260 mV, which is a favorable low working potential for a glucose biosensor, and indicates a more facile process.

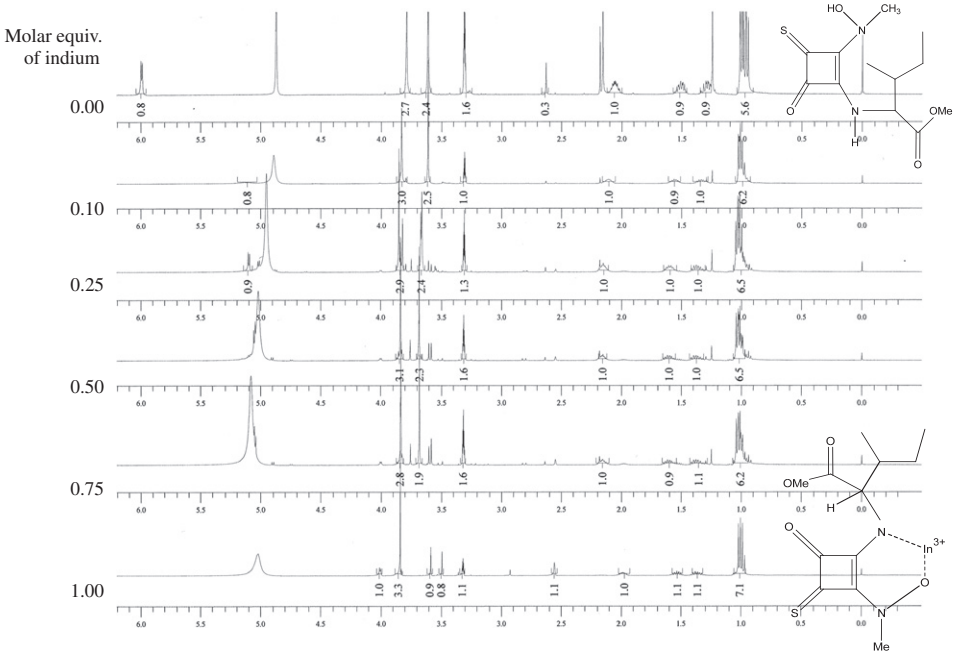
Subsequently examined was the effect of additions of D-glucose ranging from 0.01 mM to 0.1 mM to electrode 4 (Fig. 5). This resulted in increased responses as the glucose concentrations increased, at +260 mV, demonstrating a substantial lowering of the oxidation potential of hydrogen peroxide, which is evolved upon enzymatic oxidation of D-glucose by glucose oxidase. Calibration of the Pt/p(HEMA)/p(pyrrole)/SQI/GOx electrode in [bmim][BF_4], to glucose



Scan 1. ^1H NMR spectra in absence and presence of one molar equivalent of indium triflate complexed to squarate 1 to produce SQI in CD_3OD.



Scan 2. ^{1}H NMR spectra upon increasing molar equivalents of indium triflate to squarate 2 to produce SQII in $CDCl_3$.



Scan 3. ^{1}H NMR spectra upon increasing molar equivalents of indium triflate to squarate 2 to produce SQII in CD_3OD.

standards was then carried out. These time-based studies (carried out at +260 mV) gave excellent linearity ($y = 0.0257x$, $r^2 = 0.9954$) in detecting D-glucose.

This electrochemical investigation was then repeated incorporating SQII as the mediator in the gel formulation. We found that a new working potential of +200 mV resulted, making the thio analogue of the mediator an even better addition to the sensor for glucose detection.

We examined the effect of additions of D-glucose ranging from 0.01 mM to 0.07 mM to the electrode (Fig. 6). This again, resulted in increased responses as the glucose concentrations increased, but this time, at a potential of +200 mV. Calibration of the Pt/p(HEMA)/p(pyrrole)/SQII/GOx electrode in [bmim][BF_4], to glucose standards was then carried out. These time-based studies (carried out at +200 mV) gave excellent linearity ($y = 0.1759x$, $r^2 = 0.9938$) in detecting D-glucose. The optimized glucose biosensor was able to function at a much lowered potential, thereby decreasing the effect of interferents and increasing its potential use. There have been a number of reports of other glucose biosensors [13] with a similar oxidation potentials of about +200 to +300 mV. However, in these biosensors the magnitude of the current obtained per unit concentration of D-glucose was twenty times lower [14]. These

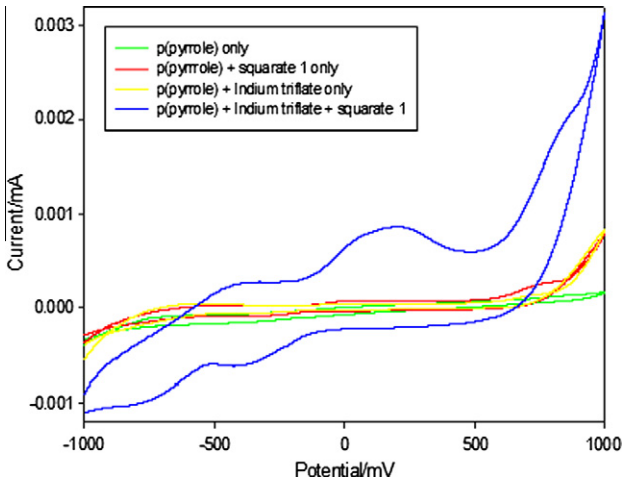


Fig. 4. Cyclic voltammograms of electrode at various stages of preparation. Experimental conditions – Temp. = 25 °C, 5 ml [bmim][PF6] in cell, scan range −1000 mV to +1000 mV, [D-glu] = 0.1 mM.

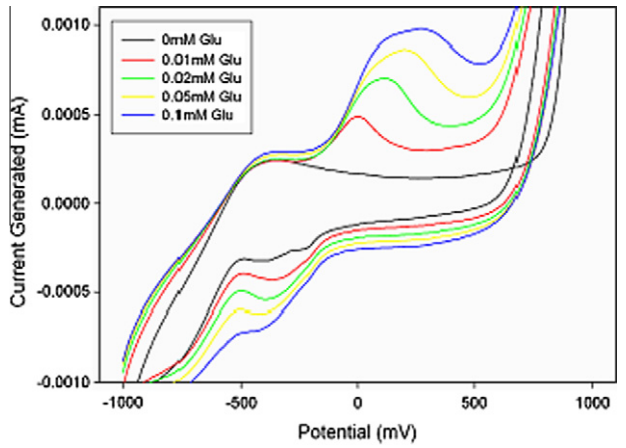


Fig. 5. CV's of electrode containing ppyrrole + SQI upon standard additions of glucose.

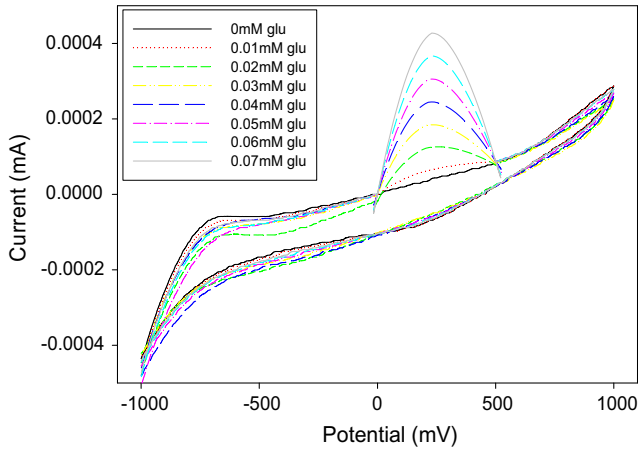


Fig. 6. CV's of electrode containing p-pyrrole + SQII upon standard additions of glucose.

newly prepared amperometric sensors has both advantages of low working potential as well as a high current output per unit mole of D-glucose.

The new gel formulation used together with [bmim][BF_4] as the solvent medium allows for high current outputs per mole of D-glucose, along with a low operational potential with a response time of 10 s. These investigations provide a secure basis for the use of squarate redox mediators and ionic liquids in gel formulations of D-glucose biosensors.

The postulated binding mode of the indium metal to the peptidic squarate (Fig 3b) evidently is one which gives rise to a highly conducting band of electrons as indicated by the enhanced functioning of the biosensor which is increased evidence for the proposed structural feasibility of the complex.

Scan 4 shows the H NMR spectra of squarate 2 in the absence and presence of one molar equivalent of indium triflate in DMSO as the solvent system. The α proton of the Ile side chain undergoes a shift of 5.982 ppm (m, 1H) to 4.018 ppm (s, 1H), while the N-methyl peak broadens, again demonstrating a complexation site at the two nitrogen atoms of the side chains.

These complexation studies were then carried out using another metal, in the form of zinc chloride, with squarate 2, in both deuterated chloroform and methanol as solvent systems (Scans 5 and 6 respectively). Similar shifts as stated for the indium complexes were again demonstrated. In $CDCl_3$, the chiral proton moves upfield from 5.867 ppm (br s, 1H) to 5.168 ppm (s, 1H), while the N-methyl protons split slightly.

There is a gradual disappearance of the protons of the –NH and –OH groups (as they are exchanged) due to the presence of a small but increasing amount of deuterated methanol in the system, as the zinc chloride does not completely dissolve in $CDCl_3$ and some CD_3OD had to have been added.

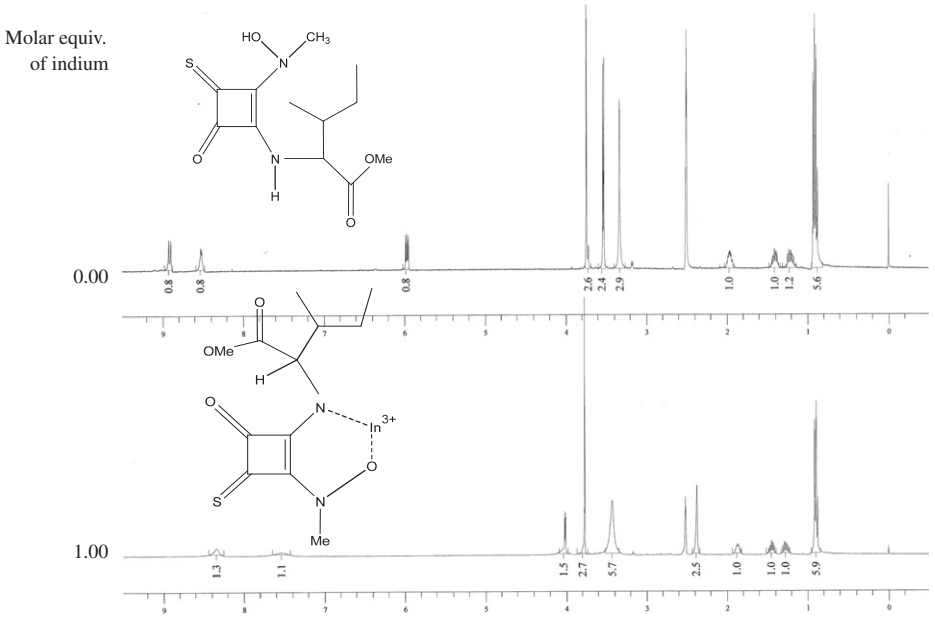
Also, due to the hygroscopic nature of zinc chloride, there is the gradual appearance of a water peak at a δ of approximately 1.6–1.8 ppm (Scan 5). In CD_3OD, the chiral proton of squarate 2 shifts from 5.997 ppm (s, 1H) to 5.226 ppm (s, 1H) in SQIII.

These studies indicate that the binding site as previously proposed of metal atoms to these peptidic squaric acid compounds and their analogues is not simply as that observed with hydroxamic acid, but possess their unique mode of complexation, regardless of the metal or solvent system. This would indicate that the design of drugs based on these squaric acid compounds, such as MMP inhibitors should focus on derivatization of the $-NR_2$ side chains of the squaric acid compounds for appropriate positioning of the metal group present in the active site of biological molecules for more effective inhibition.

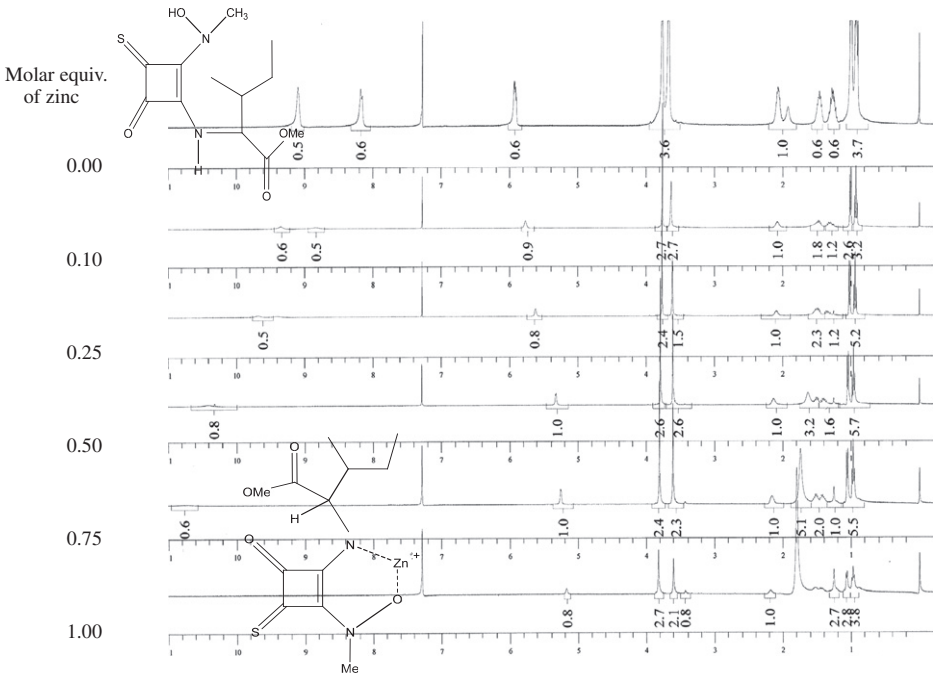
3. Experimental section

3.1. Materials

Glucose oxidase (GOx), (Type VII, E.C. 1.1.3.4. from *Aspergillus niger*, 128,000 units/g solid) and 1-butyl-3-methylimidazolium tetrafluoroborate were obtained from Sigma Chemical Co. (St. Louis, MO). Hydroxyethyl methacrylate (HEMA) was obtained from Polysciences Inc. (Warrington, PA); the crosslinking agent tetraethyleneglycol diacrylate (TEGDA), inhibitor remover columns (250 ml capacity; packed with alumina to remove phenolic inhibitor), platinum foil (0.1 mm thick), platinum wire (0.1 mm diameter), dimethoxyphenyl acetophenone (DMPA), indium (III) trifluoromethanesulfonate and pyrrole were all purchased from Aldrich Chemical Co. (Milwaukee, WI). The HEMA monomer was vacuum distilled (1.3 mmHg, 80 °C) before use. All other reagents used were of the analytical reagent grade (and distilled or recrystallized as necessary prior to use) and were obtained from BDH (Poole, UK). Cyclic voltammetry and time-based electrochemical experiments were carried out using the BAS 100B Electrochemical Analyzer. NMR data was acquired using a 400 MHz Bruckner NMR Machine.



Scan 4. ^{1}H NMR spectra in absence and presence of one molar equivalent of indium triflate complexed to squarate 2 to produce SQII in DMSO.



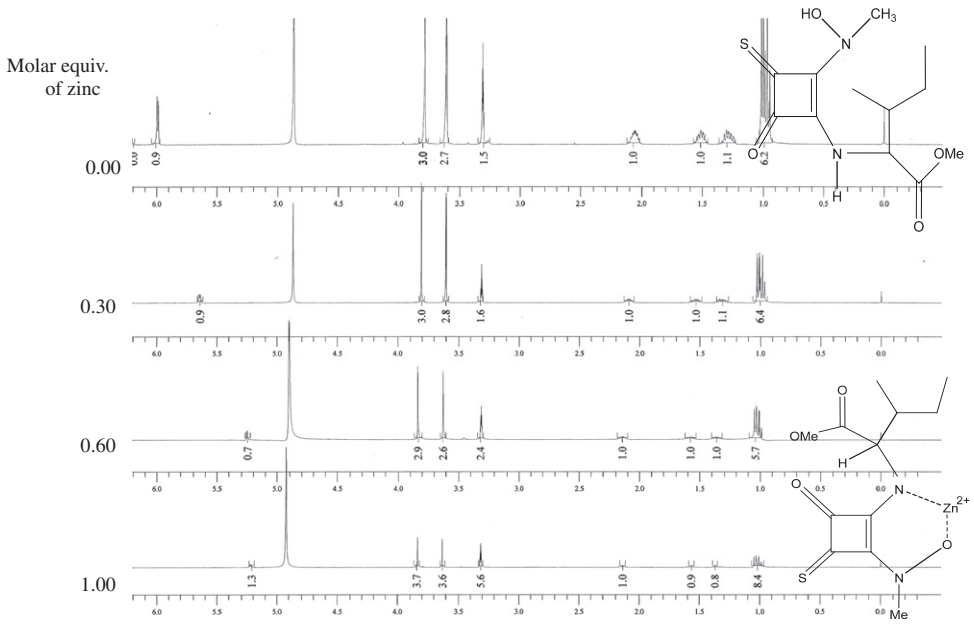
Scan 5. ^{1}H NMR spectra upon increasing molar equivalents of zinc chloride to squarate 2 to produce SQIII in $CDCl_3$.

3.1.1. Synthesis of SQI

3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione [1] (0.15 g or 5.5×10^{-4} mol) was added to an equimolar amount of $In(OTf)_3$ (0.3 g) in methanol (4 mL) and the two solids dissolved with agitation. The solution was left to stand, desiccated for 1 h, after which 2 g of DOWEX® MONOSPHERE® 550A (OH) anion exchange resin was added and gently swirled in the solution for 1 min, in order to remove any triflic acid that may have been formed. The resin was then removed by gravity filtration, and the solvent (methanol) removed under reduced pressure. ^{1}H NMR and ^{13}C NMR, analyses were then performed on the resulting compound using d^{4}-MeOH as the solvent.

3.1.2. Synthesis of SQII

3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one [1] (80 mg or 2.8×10^{-4} mol) was added to an eqimolar amount of $In(OTf)_3$ (0.157 g) in methanol (3 mL)and the two solids dissolved with agitation. The solution was left to stand for 1 h in a desiccator, after which 1.5 g of DOWEX® MONOSPHERE® 550A (OH) anion exchange resin was added and gently swirled in the solution for 1 min, in order to remove any triflic acid that may have been formed. The resin was then removed by gravity filtration, and the solvent (methanol) removed under reduced pressure. ^{1}H NMR and ^{13}C NMR analyses were then performed on the resulting compound using varying solvent systems, namely



Scan 6. ^{1}H NMR spectra upon increasing molar equivalents of zinc chloride to squarate 2 to produce SQIII in CD_3OD.

d-methanol, d-DMSO and d-chloroform. Also, ^{1}H NMR analyses of squarate 2 was recorded in the presence of different concentrations of indium triflate, in the proportions 0%, 10%, 25%, 50%, 75% and 100% (i.e. increasing mole ratio of squarate 2 to indium from 1:0 to 1:1 progressively) using d-methanol as the solvent, and then repeated using d-chloroform as the solvent. These experiments were carried out to determine if there were any observable shifting of peaks up to the point of a stoichiometric combination of the two compounds and hence shed light on the nature of the complexation between the squarate and the indium metal.

3.1.3. Synthesis of SQIII

3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one [1] (80 mg or 2.8×10^{-4} mol) was added to an equimolar amount of $ZnCl_2$ (0.038 g) in methanol (3 mL) and the two solids dissolved with agitation. The solution was left to stand for 1 h in a dessicator, after which 1.5 g of DOWEX® MONOSPHERE® 550A (OH) anion exchange resin was added and gently swirled in the solution for 1 min, in order to remove any hydrochloric acid that may have been formed. The resin was then removed by gravity filtration, and the solvent (methanol) removed under reduced pressure. ^{1}H NMR and ^{13}C NMR, analyses were then performed on the resulting compound using the solvent systems, d-methanol and d-chloroform. Also, ^{1}H NMR analyses of squarate 2 was recorded in the presence of different concentrations of zinc chloride, in the proportions 0%, 10%, 25%, 50%, 75% and 100% (i.e. increasing mole ratio of squarate 2 to zinc from 1:0 to 1:1 progressively) using d-methanol as the solvent, and then repeated using d-chloroform as the solvent. These experiments were carried out to determine if there were any observable shifting of peaks up to the point of a stoichiometric combination of the two compounds and hence shed light on the nature of the complexation between the squarate and the zinc metal.

3.2. Cleaning and preparation of platinum electrodes

Platinum electrodes (10×15 mm) were cleaned using the following standard protocol:[24] washing for 1 min each in boiling trichloroethylene, then boiling acetone, followed by sonication (1 min) in propan-2-ol and finally in deionized (DI) water. Subsequently cathodic cleaning [(-1.2 V to -2.0 V vs. SCE for ten cycles (10 s per cycle) in 0.1 M phosphate buffer (NaH_2PO_4) containing KCl (0.01 M), pH = 7.2)] was carried out followed by immersion in the cleaning agent, NH_4OH:H_2O_2:H_2O/1:1:5 v/v, for ten seconds at 60 °C. The electrode was then dried for 30 min at 60 °C. Activation of the cleaned electrode was carried out by: immersion in 10 mL of a 1% w/v sodium dithionite solution, for 30 min at 56 °C, followed by immersion in 10 mL of a 2.5% solution of $K_2Cr_2O_7$ in 15% HNO_3 for 30 min at 56 °C then rinsed with DI water (25 ml) and oven dried (30 min) at 60 °C.

Following this, an area (0.25 cm^2) was demarcated on the clean electrode surfaces using transparent scotch tape. The cleaned Pt electrode surfaces were treated with 5 mL of a 1% 3-aminopropyltrimethoxysilane (γ-APS) in dry toluene (30 min) with agitation, and subsequently cured at 110 °C for 10 min. The counter (platinum) electrode was cleaned via flaming until red hot and then washing with acetone (10 ml) and isopropanol (10 ml) successively.

3.3. Preparation of biosensors

Glucose oxidase (40 mg) was dissolved in 55 μL of HEMA and 20 μL of TEGDA. Freshly distilled pyrrole, (10 μL), was added to the resultant mixture followed DMPA (0.5 mg), and purged with argon for 5 min. The mixture (5 μL) was applied to the electroactive window and immediately irradiated with UV light (366 nm) for 45 min. The electrode was immersed in (0.4 M) solution of freshly distilled pyrrole in the ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate) (3 ml), contained in a one-compartment cell, that contained a platinum coil counter electrode and a Ag/AgCl reference electrode. A constant potential of +850 mV was applied for 100 s, affording a dark green homogenous film within the hydrogel. The resultant p(HEMA)/p(pyrrole)/GOx electrodes were washed with a small amount of ionic liquid (2 ml) and stored in the same ionic liquid. Similarly a second electrode was prepared that included the 3-(hydroxymethylamino)-2-(L-phenylalanine methyl ester)-4-thioxo-2-cyclobuten-1-one (7 mg), in the gel formulation; a third, which included indium(III) trifluoromethanesulfonate (5 mg) and a fourth which incorporated SQ1 (6 mg) and pyrrole deposition carried out as described previously.

The prepared electrodes were analyzed via cyclic voltammetry in order to determine which of the added components lowered

the working potential required for the electrochemical oxidation of hydrogen peroxide.

3.4. Amperometric response of Pt/p(HEMA)/p(pyrrole)/SQI/GOx electrode to glucose standards

A calibration plot was generated utilizing the standard addition method. To evaluate the electrode in terms of its glucose response, the electrode was made into the working electrode in a one-compartment cell with a platinum coil counter electrode and a Ag/AgCl reference electrode in 5 ml of ionic liquid. A constant potential of +260 mV was applied and the background current allowed to stabilize in order to obtain a base line reading, for compensation of background responses. D-Glucose, (0.01 ml), of a 1 M solution in ionic liquid was added to the cell (causing a 2 mM change in concentration, assuming negligible change in volume), and the response noted. This was repeated until the response to a 10 mM concentration of D-glucose was achieved.

3.5. NMR characterization of compounds

3-(hydroxymethylamino)-4-(L-isoleucine methyl ester)-3-cyclobutene-1,2-dione (squarate 1) – ^{1}H NMR (400 MHz, CD_3OD) δ 4.867 (br s, solvent), 4.801 (s, 1H), 3.771 (s, 3H), 3.482 (s, 3 H), 2.034 (m, 1H), 1.546 (m, 1H), 1.263 (m, 1H), 0.976 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 179.562, 179.161, 172.027, 166.748, 166.952, 62.534, 51.846, 40.438, 38.501, 24.942, 14.617, 10.631.

SQI – ^{1}H NMR (400 MHz, CD_3OD) δ 4.872 (br s, solvent), 4.782 (s, 1H), 4.012 (s, 1H), 3.778 (m, 3H), 3.487 (s, 1H), 3.309 (m, 1H), 2.947 (s, 1H), 2.045 (m, 1H), 1.553 (m, 1H), 1.306 (m, 1H), 0.970 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 171.897, 171.542, 170.843, 169.307, 166.496, 166.032, 125.496, 122.329, 119.163, 116.00, 62.281, 51.981, 40.514, 38.980, 24.947, 14.602, 10.707.

3-(hydroxymethylamino)-2-(L-isoleucine methyl ester)-4-thioxo-2-cyclobuten-1-one (squarate 2) – ^{1}H NMR (400 MHz, CD_3OD) δ 5.997 (s, 1H), 4.868 (br s, solvent), 3.783 (s, 3H), 3.607 (s, 3H), 2.055 (m, 1H), 1.503 (m, 1H), 1.321 (m, 1H), 0.984 (m, 6H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.051 (br s, 1H), 8.203 (br s, 1H), 7.264 (s, solvent), 5.867 (br s, 1H), 3.750 (s, 3H), 3.661 (s, 3H), 2.156 (m, 1H), 1.457 (m, 1H), 1.239 (m, 1H), 0.934 (m, 6H); ^{1}H NMR (400 MHz, ^{d6}DMSO) δ 8.920 (br s, 1H), 8.523 (br s, 1H), 5.982 (m, 1H), 3.741 (s, 3H), 3.332 (s, 3H), 2.507 (s, solvent), 1.953 (m, 1H), 1.408 (m, 1H), 1.204 (m, 1H), 0.896 (m, 6H); ^{13}C NMR (100 MHz, ^{d6}DMSO) δ 207.981, 204.168, 171.327, 60.013, 53.293, 31.936, 25.637, 15.123, 12.329.

SQII – ^{1}H NMR (400 MHz, CD_3OD) δ 5.020 (br s, solvent), 4.008 (s, 1H), 3.837 (s, 3H), 3.585 (s, 1H), 3.492 (s, 1H), 3.325 (m, 1H), 1.987 (m, 1H), 1.506 (m, 1H), 1.368 (m, 1H), 0.998 (m, 6H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.271 (s, solvent), 5.009 (s, 1H), 3.854 (s, 3H), 3.642 (s, 3H), 2.168 (m, 1H), 1.506 (m, 1H), 1.254 (m, 1H), 0.979 (m, 6H); ^{1}H NMR (400 MHz, ^{d6}DMSO) δ 8.369 (br s, 1H), 7.507 (br s, 1H), 4.018 (s, 1H), 3.770 (s, 3H), 3.428 (s, 3H), 2.521 (s, solvent), 1.839 (m, 1H), 1.447 (m, 1H), 1.257 (m, 1H), 0.914 (m, 1H); ^{13}C NMR (100 MHz, ^{d6}DMSO) δ 202.513, 196.484, 170.109, 126.311, 123.111, 119.910, 117.812, 56.912, 53.538, 36.769, 25.962, 15.090, 12.283.

SQIII – ^{1}H NMR (400 MHz, CD_3OD) δ 5.226 (s, 1H), 4.923 (s, solvent), 3.835 (s, 1H), 3.631 (s, 3H), 3.312 (s, 3H), 2.149 (m, 1H), 1.573 (m, 1H), 1.369 (m, 1H), 1.007 (m, 6H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.283 (s, solvent), 5.168 (s, 1H), 3.801 (s, 3H), 3.592 (s, 3H), 2.167 (s, 1H), 1.483 (s, 1H), 1.253 (s, 1H), 0.987 (m, 6H).

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