Exchange Reactions between Disulphides and Myocrisin: an *In Vitro* Model for a Mechanism in Chrysotherapy

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Abstract

Disodium aurothiomalate or Myocrisin has been found to react with the disulphides, 5,5′-dithio-bis-(2-nitrobenzoic acid) – commonly known as Ellmans reagent – and lipoic acid. A new polymeric species $(ESAu)_x$ is identified in solution. Two specific reactions are discussed which result in the formation of mixed disulphide with Ellmans reagent and the formation of dithiomalate with lipoic acid. The gold nuclei are transferred to the incoming moiety with the result that the disulphide linkage is opened. The importance of this reaction for cellular function is discussed.

Introduction

Disodium aurothiomalate(I) (Myocrisin) has been shown to have a specific effect in rheumatoid arthritis [1]. Model studies on the reaction of Myocrisin with simple thiols [2] indicate that the key step in its *in vivo* activity arises from an interaction with the sulphydryl group. The compound itself is polymeric in solution and in one form at least, has been shown to consist of a gold–sulphur–gold chain, with terminal thiomalate groups [3]. Throughout the polymer there are gold–gold interactions producing an A-frame arrangement [4].

The chain structure of Myocrisin, with its weak, localised interactions between thiophyllic metal centres suggested that this species may have a capacity to react with disulphides as well as thiols [2]. This hypothesis has deeper significance when the greater availability *in vivo* of disulphide linkages over thiols is considered. For this reason, a model system involving the interactions of Myocrisin with simple disulphides was studied. The disulphides chosen were 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellmans reagent) and lipoic acid. The former was chosen because the chromophore in this molecule enables the use of resonance Raman spectroscopy to provide a sensitive method of studying changes in the coordination and oxidation state of the sulphur in aqueous solution. This method in combination with ^1H nuclear magnetic resonance (NMR) provides a detailed assessment of the solution phase species formed.

Experimental

Myocrisin as an off white solid was obtained from May and Baker. It was analysed for carbon, hydrogen, sulphur and sodium (found: C, 12.86; H, 1.34; S, 7.45; and Na, 9.05% respectively). This composition was consistent with that previously reported by Sadler *et al.* [5]. Ellmans reagent (Sigma chemicals) and lipoic acid (BDH) were used without further pretreatment.

Raman spectra were recorded on an Anaspec modified Cary 81 with photon counting detection using the 457 nm line of a Spectra Physics 2020 Argon ion laser for excitation. NMR spectra were recorded on a Bruker WM 250 MHz Aspect 2000 spectrometer. All NMR spectra were recorded in ^2H_2O/NaCl (0.154 M)/Na_2HPO_4 (0.125 M) solution adjusted to pH 7.4.

Results and Discussion

The partial structural analysis by Elder [3, 4], clearly shows the molecule to be an open chain polymer in solution. There is considerable difficulty in re-crystallising this molecule, in part because of the glycerol (~thiomalate:glycerol 3:1), which is associated with it. Due to the variable glycerol and water content of the commercial material the standard carbon, hydrogen, nitrogen analysis can be manipulated to confirm a variety of structures [2–5]. We have determined using the available data and NMR that the chain length in Myocrisin contains between seven and nine gold atoms, with an experimental preference for eight gold atoms to nine thiomalates. This description will be used throughout the manuscript. This is consistent with the structure presented by Elder [3, 4], in that a centre of symmetry has been introduced into the molecule (Fig. 1).

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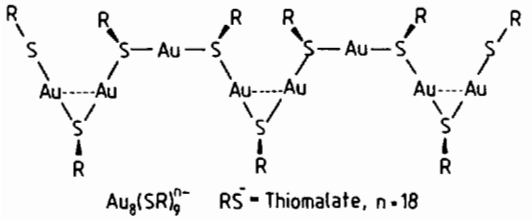


Fig. 1. The polymeric structure of disodium aurothiomalate-(I), Myocrisin ($Na_{18}Au_8ThM_9$: ThM = thiomalate). The glycerol and water content of the material are omitted.

On the addition of small amounts of a solution of Ellmans reagent to a solution of Myocrisin an intense colour is formed. Raman spectroscopy detects this change as the formation of a new signal at 1333 cm^{-1} (*c.f.* Ellmans reagent, ESSE, at 1348 cm^{-1} and Ellmans anion, ES^-, at 1325 cm^{-1}: Fig. 2). We have assigned this new species to a ES–Au moiety. There is no evidence in the spectrum to support the formation of the ES^- anion during the initial reactive step.

Proton NMR (Fig. 3) using more concentrated solutions, but with similar molar ratios, indicates the formation of two new products which have ES components included in the structure. One set of ES resonances has the same line width characteristics as the Myocrisin polymer and the other set are sharp. Thus we conclude that a thiol exchange reaction between Myocrisin and Ellmans reagent has occurred. Replacement of the terminal groups on the polymer by Ellmans reagent will produce two species; mixed disulphide (ESThM; ThM = thiomalate) and a polymer capped by ES moieties. This will give rise to two sets of resolved signals in the low field region (6.8–8.0 ppm) of the NMR (Fig. 3), with the overall reaction shown in eqn. (1).

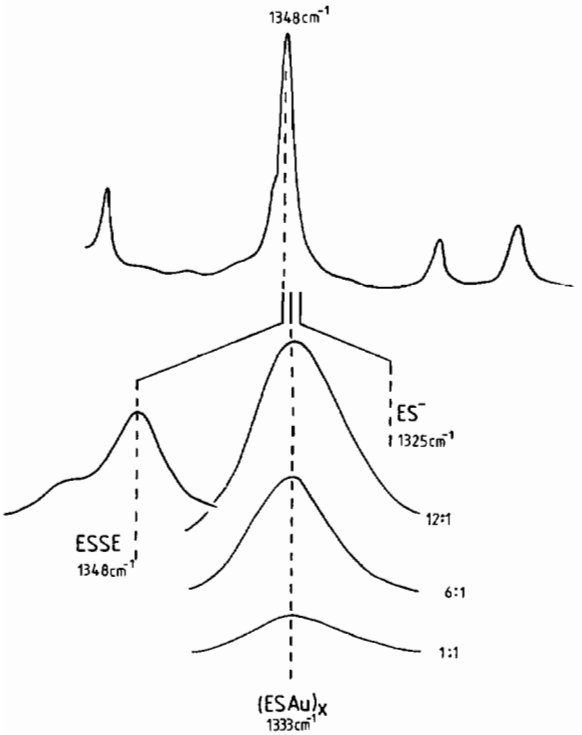


Fig. 2. Raman spectrum of Ellmans reagent illustrating the strong band at 1348 cm^{-1} due to the symmetric N–O stretch of the nitro-group. Spectra on an expanded wavelength scale for the titration of Myocrisin with Ellmans reagent are given below. No ES^- is observed and the intensity of the $(ESAu)_x$ peak increases with gold concentration. Ratios are given for atoms of gold to molecules of Ellmans reagent.

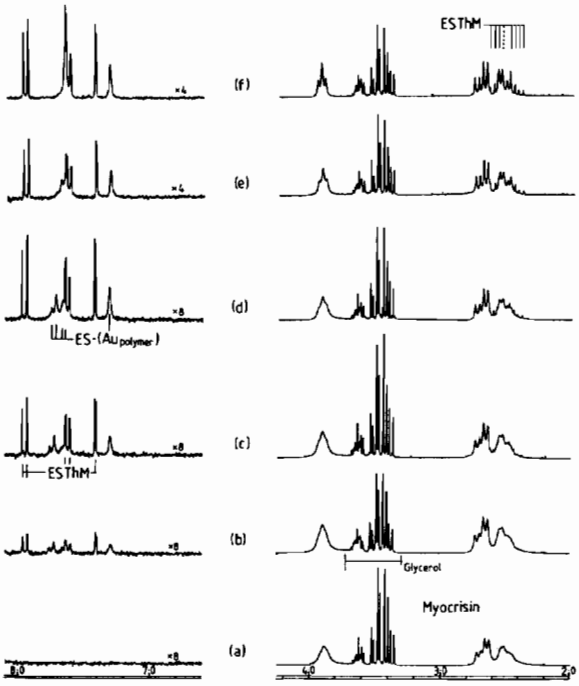


Fig. 3. Proton NMR of the reaction of Myocrisin with Ellmans reagent (ESSE). (a) Myocrisin, 10.0 mg in 0.5 ml of buffer; (b) +0.2 mg ESSE; (c) +0.4 mg ESSE; (d) +0.6 mg ESSE; (e) +0.8 mg ESSE; and (f) +1.0 mg ESSE. The Ellmans reagent was added as a solution in $^2H_2O/NaCl/Na_2HPO_4$ at pH 7.4. The low field regions are multiplied by the factors shown.

$$2ESSE + ThM–(Au_8ThM_7)–ThM \longrightarrow ES–(Au_8ThM_7)–SE + 2ESThM \quad (1)$$

As the Ellmans reagent concentration is increased in solution the intensity of the 1333 cm^{-1} line diminishes. There is no evidence in the resonance Raman spectra to support the presence of appreciable concentrations of ESSE or ES^- in the solution as both give rise to signals using 457 nm irradiation. The mixed disulphide is expected to have an electronic spectrum which lies further to the UV and is unlikely to be observed by resonance Raman at these concentrations. However, the identity of this species is provided by ^1H NMR where by treating thiomalate with

both Ellmans reagent and sodium periodate it is possible to generate both the mixed disulphide and the dithiomalate species separately. A comparison of the NMR spectra (Figs. 3, 4) clearly identifies the species to be mixed disulphide.

Under similar circumstances in the ^1H NMR, increased Ellmans reagent concentration causes the polymer resonances to both resolve and collapse (Fig. 5). This suggests that the polymer is being degraded, however during this process it also undergoes a reorganisation in its structure. These observations are explained by allowing terminal exchange of the thiomalate groups to proceed rapidly and preferentially (eqn. (1)). The intra-gold associations envisaged in Fig. 1 confer a degree of stability on the polymer. However at higher Ellmans reagent concentrations these weak bonds break, allowing the molecule a further degree of mobility and therefore a further degree of resolution in the NMR line shape prior to overall degradation (eqn. (2)) which is slow compared to terminal group exchange.

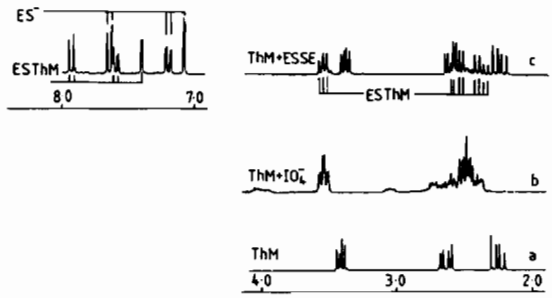


Fig. 4. The oxidation of thiomalate (a); by periodate producing dithiomalate (b) and by Ellmans reagent to produce mixed disulphide (c).

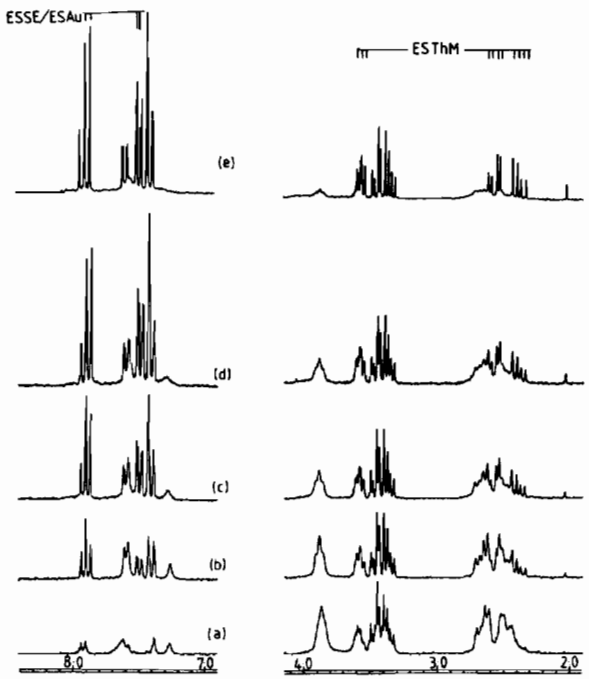


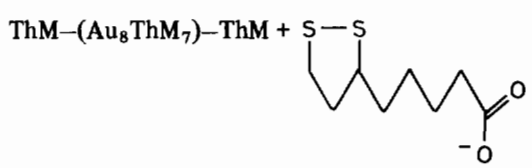
Fig. 5. Proton NMR of the reaction of Myocrisin with Ellmans reagent (ESSE). (a) Myocrisin, 10.0 mg in 0.5 ml of buffer + 1.0 mg of ESSE; (b) 3.0 mg ESSE; (c) +5.0 mg ESSE; (d) +7.0 mg ESSE and (e) +9.0 mg ESSE. The complete reaction is slow in the closing stages with the end point occurring at <9.0 mg (22.7 μmol) of ESSE. The solutions shown in Fig. 3 and here are different in origin.

$$2[ES{-}(Au_8ThM_7){-}SE] + 13ESSE \longrightarrow 16/x(ESAu)_x + 14ESThM \quad (2)$$

That there are only two ES containing species in the low field region of the NMR spectra is explained by the presence of fast exchange between ESSE and the polymeric $(ESAu)_x$, thus suggesting that the coordination number of the gold is always two. This behaviour is consistent with that previously reported [2] for the coordination number achieved by the gold nucleus in this complex during fast exchange with simple thiols. The overall, general, reaction (eqn. (3)) plus the end point of the titration (Fig. 4e, f) provides a good approximation to the overall maximum chain length of the polymer (8:9 or 9:10, gold:thiomalate).

$$2[ThM(Au_nThM_n)] + (2n + 1)\,ESSE \longrightarrow 2n ESAu + 2(n + 1)ESThM \quad (3)$$

The reaction of Ellmans reagent with Myocrisin provides a useful chemical model, but ESSE is an aromatic disulphide with a rather different reactivity to biologically available disulphides. To analyse this process further we reacted Myocrisin with lipoic acid, a naturally occurring microbial disulphide of immediate importance to the cellular redox status. Here a slow direct reaction is observed (Fig. 6), which indicates that a complete exchange has occurred producing dithiomalate and not as is observed above, the mixed disulphide (eqn. (4)). The resonances from the lipoic acid become poorly resolved suggesting that they have replaced the thiomalates in the polymer structure. The multi bonding arrangements which this species can adopt will contribute significantly to the form of the complex and the partially resolved nature of certain portions of the spectra.



$$\longrightarrow (ThM)_2 + \text{Au:Lipoic Polymer} \quad (4)$$

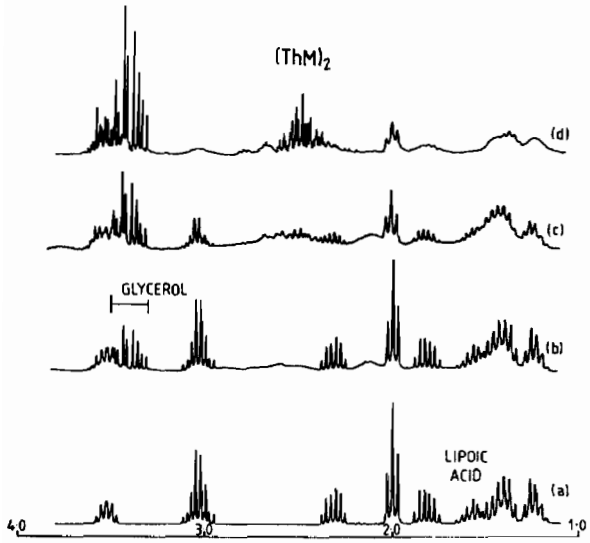


Fig. 6. Proton NMR of the reaction of Myocrisin (8.8 mg) with excess lipoic acid (8.7 mg). (a) Lipoic acid; (b) immediately after the addition of Myocrisin; (c) after 1 week; and (d) after 2 weeks. The absence of Myocrisin signals (3.5–3.3 ppm) in (b) compared to the prominence of the glycerol (*c.f.* Fig. 3) suggests that the terminal exchange process observed with Ellmans reagent (eqn. (1)) has occurred before the spectrum can be acquired.

Thus the reactions of Myocrisin with these two disulphides are different but complete. It suggests that where disulphides are exposed to Myocrisin *in vivo*, a similar reaction to that of lipoic acid may be possible. This type of reaction has obvious implications for the changes in protein structure which are envisaged as being important in certain diseases such as rheumatoid arthritis [1], where oxidative stress is believed to be responsible for an increase in disulphide cross linkages in proteins. On reaction with Myocrisin, the disulphides will be opened. However, the small polymer formed can then be transferred to other disulphides by intramolecular shift reactions targeting the drug on potentially important sites deep in the protein channels.

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