Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 11, pp. 1353-1356, 1994

0960-894X/94 $7.00+0 00

0960-894X(94)00155-3

THE SELENIUM MEDIATED DE-IODINATION OF IODOPHENOLS: A MODEL FOR THE MECHANISM OF 5' THYRONINE DE-IODINASE.

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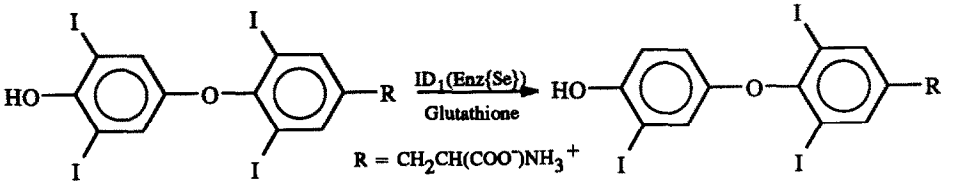
ABSTRACT

The de-iodination of aryl-phenols by benzeneselenol is investigated as a model of the seleno-protein, 5'-thyronine de-iodinase. The results suggest that the mechanism involves the stabilisation of the ketonic tautomer of thyroxine (T_4) prior to the abstraction of an iodine by RSe^-. The RSeI formed can then be reduced by a thiol co-factor to regenerate the active enzyme.

INTRODUCTION

It is now recognised that there are three different classes of selenium containing biomolecules; the storage protein seleno-protein-P and the enzymes glutathione peroxidase (GSP_x) and type I thyronine de-iodinase (ID-1) [1]. The latter enzyme is central to the de-iodination of thyroxine (T_4) which produces the active hormone tri-iodothyronine (T_3). Although, it is recognised that a selenium nucleus is at the active site of this enzyme, little is known about the mechanism employed by ID-1 apart from its use of glutathione as a co-factor (equation 1) [1-3].



-(1)

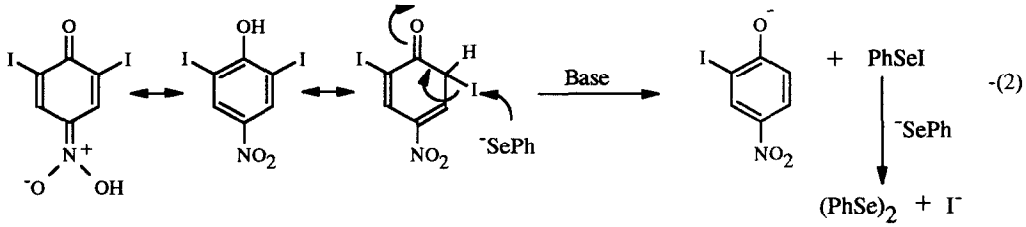
T_4 Thyroxine T_3 Tri iodothyronine

Other factors, other than the presence of a selenium centre at the active site of ID-1, must be involved in the overall reaction mechanism. If the de-iodination reaction were dependent solely on the presence of a redox active selenium centre then GSP_x, which contains selenium and also employs glutathione as a co-factor, might be expected to show some residual de-iodinase activity, which is not the case.

Due to the small amounts of protein available, a complete structural characterisation of ID-1 by NMR or X-ray methods has yet to be reported. As such, we have used chemical models in an attempt to explore the various facets of the selenium mediated de-iodination reaction. To try and marry the chemical models with the in-vivo chemistry we have also investigated the structure of thyroxine using AM1 calculations.

Chemical Models

Simple di-iodophenols; 4-amino-2,6-di-iodophenol, 2,6-di-iodo-tyrosine, 4-nitro-2,6-di-iodophenol, were used as chemical models of the salient portion of thyroxine which is de-iodinated by ID-1. The different electron withdrawing/accepting properties of the functional groups para to the phenolic function help clarify any involvement of the ether bridge in thyroxine in the reaction. The conditions employed were consistent with a previous study on the de-iodination of alkylketo-iodides [4] with the products being identified here by TLC and GC-MS. These experiments indicated that with these aryl species only when there was a strong electron withdrawing group present (i.e. $-NO_2$) did de-iodination take place [5]. This suggests that the ketonic resonance form of the di-iodophenol plays an important role in the reaction (equation 2). As the equivalent keto- resonance form cannot be sustained to any great extent by either 4-amino-2,6-di-iodophenol or 2,6-di-iodo-tyrosine, facile de-iodination under mild conditions by benzeneselenol is not as favoured.



-(2)

Although this reaction can be effected using thiophenol under similar conditions, unlike the recent report of the de-iodination of iodohistidenes we were unable to effect the reaction with mercaptoethanol [6]. Subsequently, by using a 10 fold excess of mercaptoethanol (RS^-) it was possible to increase the reaction turnover by reductively regenerating the benzeneselenol (equation 3).

$$RS^- + PhSe\text{-}SePh \longrightarrow RS\text{-}SePh + PhSe^- \qquad -(3)$$

Theoretical Models

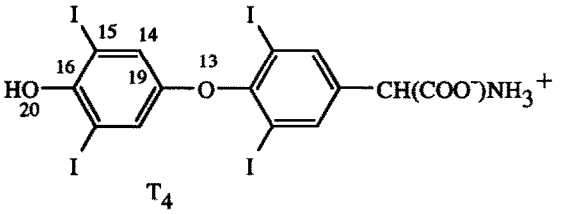
Although the model substrates suggests that the mechanism may utilise the keto form of the hormone, there was some concern as to how this tautomer was generated and stabilised in-vivo. AM1 calculations [7] were carried out initially on thyroxine using the reported X-ray structure [8] as a basis set and a model complex (T_m) methylated at the bridging ether linkage and deprotonated at the phenolic function (figure 1). This latter structure was used to mimic the effect of a strong electron withdrawing group in the vicinity of the ether linkage in the active site of the enzyme.

The calculations show that when electron density is removed from the aryl ring (i.e. the O-methylated form mT_4), the bond length of the phenolic C_{16}-O_{20} is significantly shorter than found for thyroxine indicating that a keto function may be present. Other bond lengths are also significantly changed, C_{14}-C_{15} being shorter with a concomitant lengthening of C_{15}-C_{16} and C_{14}-C_{19} again confirming a change towards the keto form.

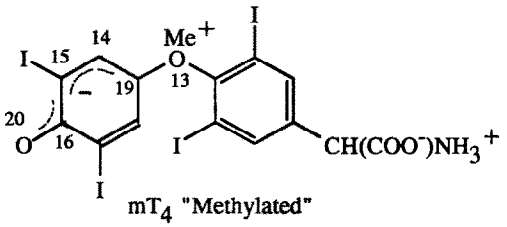
Thus the key tautomeric form of thyroxine required by the mechanism above (equation 2) would be favoured if thyroxine, on binding to the active site of ID-1, was subjected to an environment which facilitated the deprotonated of the phenolic function and the removal of electron density via an interaction with oxygen-13 in the ether bridge.

C_x-C_y pm	T_4	mT_4
14 15	139.1	136.0
15 16	141.1	146.9
14 19	139.7	141.8

C_x-O_y pm	T_4	mT_4
19 13	140.4	142.1
16 20	136.9	124.3



T_4



mT_4 "Methylated"

Figure 1. The structures and key bond lengths of thyroxine (T_4) and a model of the ketonic form (mT_4) derived from AM1 Calculations. For the purpose of the calculations oxygen-13 was methylated to mimic the generation of positive charge in the vicinity of the ether linkage.

The mechanism proposed above (equation 2) and the AM1 calculations support the hypothesis that a selenium alone cannot effect the reaction and that an important binding site in the vicinity of the ether linkage exists which activates the hormone by stabilising the keto-form. This chemistry sets ID-1 apart from GSP_x which is incapable of activating the hormone.

That we can achieve limited "catalysis", by reducing the diselenide product using simple aliphatic thiols does not allow us to make any assumptions regarding the role of glutathione. Apart from its action as a reducing agent, it unclear if the $RSeI^*$ forms directly and is then reduced by free glutathione or whether there is an active cysteinyl function in the protein which effects a sulpho-seleno linkage which is reduced by glutathione.

Using this information it is possible to construct a plausible mechanism which represents the key steps in the enzyme cycle of ID-1 (figure 2). Although the cycle below has some similarities to the two step "ping-pong" mechanism reported by Berry et al. [3], it more closely resembles the mechanism involved in the GSPx

cycle, which allows for a more correct description of the chemistry occurring at the selenium centre. However, the key difference between ID-1 and GSPx is believed to be the ability of ID-1 to enter into a non-selenium based interaction with the hormone in the vicinity of the ether linkage, inducing a rearrangement (phenol→keto) prior to the selenium mediated de-iodination.

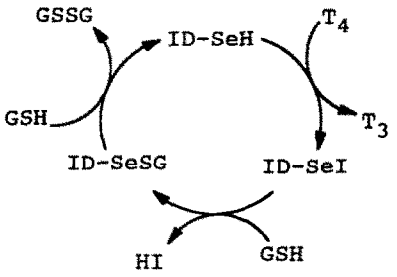


Figure 2: The proposed "catalytic" cycle for the de-iodination reaction effected by ID-1

GSH = Glutathione

* RSeI can be considered as formally containing I^+ as postulated elsewhere [2].

As the reaction can be effected using both benzeneselenol and thiophenol [6] it could be implied that this mechanism is a plausible explanation of the mechanism of both type I and the non-selenium based type II de-iodinase enzymes [9]. Similarly the type II enzyme would require a thiolate co-factor (glutathione) to regenerate the active thiolale moiety.

ACKNOWLEDGEMENTS:

We would like to thank the Wellcome trust for a vacation scholarship (CB), M.J.Stewart and S.Gibson (Glasgow Royal Infirmary) for assistance with GC-MS and Dr P.Bladon (Strathclyde Univ) for help with the AM1 calculations.

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5. The di-iodophenols (1 mmole) were stirred in ethanol (15 ml) together with anyhdrous potassium carbonate (1 mmole). The potassium carbonate does not dissolve completely but the solution turns yellow-orange. The solution was de-gassed using a stream of nitrogen prior to the dropwise addition of benzeneselenol (2 mmole). The mixture was refluxed under a nitrogen atmosphere for 12 hours whereupon it turns orange. The mixture was analysed directly by TLC (chloroform) and by GC-MS (Fisons Instrument GC-800 series with MD 800 Mass spectrometer; Column: Fisons Instruments phase DB-1 30cm x 0.32mm, film thickness 0.25 microns).
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7. The calculations were carried out on a Silicon Graphics 4020 computer. Structures were built using INTERCHEM and then optimised using PIFF or MINIMAX before final optimisation with MOPAC 6.0.
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(*Received in USA* 2 *December* 1993; *accepted* 25 *April* 1994)