*Inorganica Chimica Acta*, 66 (1982) L21–L22




# Differences in the Reaction of d-Penicillamine and l-Cysteine with 5,5-dithiobis(2-nitrobenzoic acid). A Model for some *in vivo* Differences?

J. C. BANFORD, D. H. BROWN, C. J. McNEIL and W. E. SMITH

*Department of Pure and Applied Chemistry, University of Strathclyde. Thomas Graham Building, Cathedral Street, Glasgow, G1 1XL, U.K.*

Received September 5, 1981

d-Penicillamine (dimethyl cysteine) was first developed as a drug to remove excess copper from patients with Wilson's disease [1] and it is indeed a good metal-complexing agent both *in vitro* and *in vivo* [2]. However, the action of d-penicillamine is more complex than at first supposed. For example, it is an effective therapeutic agent in the treatment of rheumatoid arthritis [3] and it is known to affect thiol/disulphide equilibria in this therapy [4, 5].

A spectrophotometric titration using the formation of $ES^-$ as an indicator was carried out for l-cysteine and d-penicillamine. Two mol of cysteine and one mol of penicillamine per mol of ESSE were required for complete reaction (Fig. 1). Some other primary thiols, namely, glutathione, β-D-thioglucose and thioglucose tetraacetate, all reacted in the same way as cysteine, whereas the secondary thiol, thiomalic acid, reacted in the same way as penicillamine.

A standard method of analysis for thiol in biological systems is by reaction with Ellman's reagent [6] – 5,5′-dithiobis(2-nitrobenzoic acid) (ESSE). This produces an aromatic thiol which is ionised at pH 7.6 and has a characteristic absorption band in this form which can be used in a spectrophotometric analysis procedure.

$$RSH + ESSE \rightarrow RSSE + ESH \quad (1)$$

$$ESH \rightarrow ES^- + H^+ \quad (2)$$

It is possible that this reaction could go further,

$$RSH + RSSE \rightarrow RSSR + ESH \quad (3)$$

The reason for the different behaviour between the two groups of thiols is not immediately obvious, and both steric and electronic effects may play a part. ESSE itself does not react readily with many partially protected thiol groups which are accessible to other reagents [7]. For example, there is one thiol group on haemoglobin which is accessible to cysteine and with which ESSE does not react. This observation and the apparent differentiation into primary and other thiols would both suggest that the steric effect may predominate, although this is not very obvious from models of these molecules.

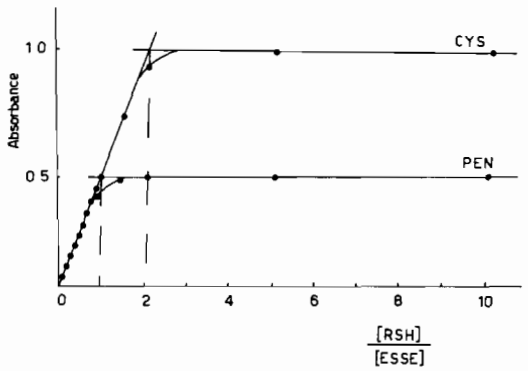


Fig. 1. A comparison of the spectrophotometric titration of cysteine and penicillamine with Ellman's reagent. The abscissa is expressed as a molar ratio.

There are, however, implications for the action of penicillamine in rheumatoid arthritis and other diseases. In a practical sense, the standard procedure of using an excess of ESSE in thiol determinatins [6] is obviously essential for reliable analysis and the use of spectroscopic titrations in conjunction with another method of thiol quantification such as radioactive labelling may help to characterise the nature of the thiol present. In a fundamental sense, cysteine in plasma is present mainly as cystine (RSSR) or complexed to metals. Penicillamine, however, is more likely to be present as a mixed disulphide bound to other naturally occurring thiols. Caeruloplasmin, the main oxidase in plasma, is a specific substrate for the oxidation of cysteine and also oxidises penicillamine more slowly [8]. It is raised in inflammation and almost absent in Wilson's disease. Thus, in inflammatory conditions, the most likely forms of penicillamine to exist for any length of time are a variety of mixed disulphides. They may well have a different and wider distribution *in vivo* than does cystine which, at normal body pH's, is rather insoluble and certainly much less soluble than the mixed disulphide of penicillamine and cysteine.

## Acknowledgements

We thank the Medical Research Council for a research assistanship and Organon Laboratories and the Science Research Council for a CASE award.

## References


1 J. M. Walshe, *Lancet, 1,* 70 (1956).
2 J. C. Crawhall, D. Lecavalier and P. Ryan, *Biopharmaceutics Drug. Dispos., 1,* 73 (1979).
3 T. E. N. Feltkamp (ed.), *Scand J. Rheumatol., Supp 28,* (1978).
4 M. Haataja, M. Nissila and H. M. Ruutsalo, *Scand. J. Rheumatol., 7,* 212 (1978).
5 A. H. Gillan and N. D. Hall, *J. Pharm. Pharmacol., 31,* 676 (1979).
6 G. L. Ellman, *Arch. Biochem. Biophys., 82,* 70 (1959).
7 R. I Weed, *J. Gen. Physiol., 45,* 395 (1962).
8 V. Albergoni and A. Cassini, *Bull. Mol. Biol. Med., 3,* 79 (1978).