

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Aldehyde dehydrogenase and
disulfiram

by Trevor M. Kitson, M.A., D.Phil.

A collection of published papers presented in
application for the degree of Doctor of Science

Massey University

New Zealand

1991

Contents

Papers dealing with aldehyde dehydrogenase and/or disulfiram

1. K.E. Crow, T.M. Kitson, A.K.H. MacGibbon & R.D. Batt. "Intracellular localisation and properties of aldehyde dehydrogenases from sheep liver." *Biochim. Biophys. Acta* 1974, **350**, 121-128.
2. T.M. Kitson. "The effect of disulfiram on the aldehyde dehydrogenases of sheep liver." *Biochem. J.* 1975, **151**, 407-412.
3. T.M. Kitson. "The effect of some analogues of disulfiram on the aldehyde dehydrogenases of sheep liver." *Biochem. J.* 1976, **155**, 445-448.
4. T.M. Kitson. "Reinvestigation of the chemical reaction between disulfiram and ethanol." *J. Stud. Alcohol* 1977, **38**, 1771-1772.
5. T.M. Kitson. "On the probability of implanted disulfiram's causing a reaction to ethanol." *J. Stud. Alcohol* 1978, **39**, 183-186.
6. T.M. Kitson. "Studies on the interaction between disulfiram and sheep liver cytoplasmic aldehyde dehydrogenase." *Biochem. J.* 1978, **175**, 83-90.
7. T.M. Kitson. "Disulfiram and cigarettes." *Alcoholism: Clin. Exp. Res.* 1978, **2**, 396-398.
8. T.M. Kitson. "2,2'-Dithiodipyridine activates aldehyde dehydrogenase and protects the enzyme against inactivation by disulfiram." *Biochem. J.* 1979, **183**, 751-753.
9. F.M. Dickinson, G.J. Hart & T.M. Kitson. "The use of pH-gradient ion-exchange chromatography to separate sheep liver cytoplasmic aldehyde dehydrogenase from mitochondrial enzyme contamination, and observations on the interaction between the pure cytoplasmic enzyme and disulfiram." *Biochem. J.* 1981, **199**, 573-579.

10. T.M. Kitson. "The inactivation of aldehyde dehydrogenase by disulfiram in the presence of glutathione." *Biochem. J.* 1981, **199**, 255-258.
11. T.M. Kitson. "Purification of cytoplasmic aldehyde dehydrogenase by covalent chromatography on reduced thiopropyl-Sepharose 6B." *J. Chromatog.* 1982, **234**, 181-186.
12. T.M. Kitson. "Further studies of the action of disulfiram and 2,2'-dithiodipyridine on the dehydrogenase and esterase activities of sheep liver cytoplasmic aldehyde dehydrogenase." *Biochem. J.* 1982, **203**, 743-754.
13. T.M. Kitson. "The activation of aldehyde dehydrogenase by diethylstilboestrol and 2,2'-dithiodipyridine." *Biochem. J.* 1982, **207**, 81-89.
14. T.M. Kitson & K.E. Crow. "Activation of aldehyde dehydrogenase by diethylstilbestrol." In *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase*, ed. Weiner & Wermuth. 1982, Liss, New York, pp. 37-52.
15. T.M. Kitson. "Mechanism of inactivation of sheep liver cytoplasmic aldehyde dehydrogenase by disulfiram." *Biochem. J.* 1983, **213**, 551-554.
16. T.M. Kitson. "The time course of the interaction of sheep liver cytoplasmic aldehyde dehydrogenase with 2,2'- and 4,4'- dithiodipyridine: a comparison with the action of disulfiram." *Arch. Biochem. Biophys.* 1984, **234**, 487-496.
17. T.M. Kitson & K.M. Loomes. "The reaction of cytoplasmic aldehyde dehydrogenase with various thiol-modifying reagents." In *Enzymology of Carbonyl Metabolism 2: Aldehyde Dehydrogenase, Aldo/Keto Reductase, and Alcohol Dehydrogenase*, ed. Flynn & Weiner. 1985, Liss, New York, pp. 43-56.
18. T.M. Kitson & K.M. Loomes. "Modification of thiol groups in cytoplasmic aldehyde dehydrogenase." *Alcohol* 1985, **2**, 97-101.

19. T.M. Kitson. "High concentrations of aldehydes slow the reaction of cytoplasmic aldehyde dehydrogenase with thiol group modifiers." *Biochem. J.* 1985, **228**, 765-767.
20. T.M. Kitson. "Effects of diethylstilbestrol, 2,2'-dithiodipyridine and chloral hydrate on the esterase activity of sheep liver cytoplasmic aldehyde dehydrogenase." *Biochemistry* 1986, **25**, 4718-4724.
21. K.M. Loomes & T.M. Kitson. "Aldehyde dehydrogenase catalyses acetaldehyde formation from 4-nitrophenyl acetate and NADH." *Biochem J.* 1986, **238**, 617-619.
22. T.M. Kitson. "Effect of disulfiram on the pre-steady-state burst in the reactions of sheep liver cytoplasmic aldehyde dehydrogenase." *Biochem. J.* 1987, **248**, 989-991.
23. T.M. Kitson. "Kinetics of p-nitrophenyl pivalate hydrolysis catalysed by cytoplasmic aldehyde dehydrogenase." *Biochem J.* 1988, **257**, 573-578.
24. T.M. Kitson. "The action of cytoplasmic aldehyde dehydrogenase on methyl p-nitrophenyl carbonate and p-nitrophenyl dimethylcarbamate." *Biochem. J.* 1988, **257**, 579-584.
25. T.M. Kitson. "The binding of NADH to cytoplasmic aldehyde dehydrogenase after modification with p-nitrophenyl dimethylcarbamate." *Biochem. J.* 1988, **257**, 585-590.
26. K.M. Loomes & T.M. Kitson. "Reaction between sheep liver mitochondrial aldehyde dehydrogenase and various thiol-modifying reagents." *Biochem. J.* 1989, **261**, 281-284.
27. K.M. Loomes & T.M. Kitson. "Studies of the action of symmetrical and mixed disulphide thiol-modifying reagents on the esterase activity of cytoplasmic aldehyde dehydrogenase." *Biochem. Biophys. Acta* 1989,

998, 1-6.

28. T.M. Kitson, J.P. Hill & G.G. Midwinter. "Identification of a catalytically essential nucleophilic residue in cytoplasmic aldehyde dehydrogenase." *Biochem J.* (in press)
29. T.M. Kitson. "Effect of some thiocarbamate compounds on aldehyde dehydrogenase and implications for the disulfiram ethanol reaction." *Biochem J.* (in press)

Other compounds with a disulfiram-like effect.

30. T.M. Kitson & K.E. Crow. "Studies on possible mechanisms for the interaction between cyanamide and aldehyde dehydrogenase." *Biochem. Pharmacol.* 1979, **28**, 2551-2556.
31. T.M. Kitson. "Methyltetrazolethiol and the Antabuse reaction." *Lancet* 1984, **II**, 1338.
32. T.M. Kitson. "The effect of 5,5'-dithiobis-(1-methyl-tetrazole) on cytoplasmic aldehyde dehydrogenase and its implications for cephalosporin-ethanol reactions." *Alcoholism: Clin. Exp. Res.* 1986, **10**, 27-32.
33. T.M. Kitson. "Inactivation of aldehyde dehydrogenase by a putative metabolite of cefamandole." *Infection* 1986, **14**, 44-45.
34. T.M. Kitson. "Aldehyde dehydrogenase and cefamandole." *Infection* 1986, **14**, 46-47.

Reviews

35. T.M. Kitson. "The disulfiram-ethanol reaction; a review." *J. Stud. Alcohol* 1977, **38**, 96-113.
36. T.M. Kitson & L. Bieder. "Drugs which interfere with ethanol metabolism." *N. Z. Med. J.* 1977, **86**, 135-137.
37. T.M. Kitson. "Disulfiram-ethanol reaction." *Psychiat. Digest* 1977 (June) p. 55.
38. T.M. Kitson. "Exorcising the demon drink." *New Scientist* 1978, **79**, 195-197.

39. T.M. Kitson. "The dithiocarbamates - interesting, versatile and neglected." *Educ. in Chem.* 1985, **22**, 43-45.
40. T.M. Kitson. "The effect of cephalosporin antibiotics on alcohol metabolism: a review." *Alcohol* 1987, **4**, 143-148.
41. T.M. Kitson. "Disulfide interchange reactions; an enzymic case study." *J. Educ. Chem.* 1988, **65**, 829-832.

Associated chemical papers

42. T.M. Kitson. "Action of nucleophiles on tetramethyl-thiuram monosulphide." *Perkin I, J. Chem. Soc.* 1977, 565-566.
43. T.M. Kitson. "Mechanism of the formation of ethylene bis(dimethyldithiocarbamate) from tetramethyl-thiuram monosulphide and sodium 2-hydroxyethane-thiolate." *Perkin I, J. Chem. Soc.* 1980, 1724-1725.
44. C.A. Bishop, T.M. Kitson, D.R.K. Harding & W.S. Hancock. "Investigation of the isolation of a mixed disulphide, diethylcarbamoyl 2-pyridyl disulphide, by analytical and preparative high-performance liquid chromatography." *J. Chromatog.* 1981, **208**, 141-147.
45. T.M. Kitson & K.M. Loomes. "Synthesis of methyl 2- and 4-pyridyl disulfide from 2- and 4-thiopyridone and methyl methane-thiosulfonate." *Anal. Biochem.* 1985, **146**, 429-430.

Early papers, before coming to Massey

46. T.M. Kitson & J.R. Knowles. "Evidence for an acyl-pepsin intermediate: a re-appraisal." *Chem Commun.* 1970, 361-362.
47. J.R. Knowles, R.S. Bayliss, A.J. Cornish-Bowden, P. Greenwell, T.M. Kitson, H.C. Sharp & G.B. Wybrandt. "Towards a mechanism for pepsin."

In Structure-Function Relationships of Proteolytic Enzymes, ed. Desnuelle, Neurath & Ottesen. 1970, Munksgaard, Copenhagen, pp. 237-250.

48. T.M. Kitson & J.R. Knowles. "The inhibition of pepsin-catalysed reactions by structural and stereochemical product analogues." *Biochem. J.* 1971, **122**, 241-247.
49. T.M. Kitson & J.R. Knowles. "The pathway of pepsin-catalysed transpeptidation." *Biochem. J.* 1971, **122**, 249-256.
50. T.M. Kitson & J.R. Knowles. "The effect of arginine modification on the pH dependence of pepsin activity." *FEBS Letters*, 1971, **16**, 337-338.

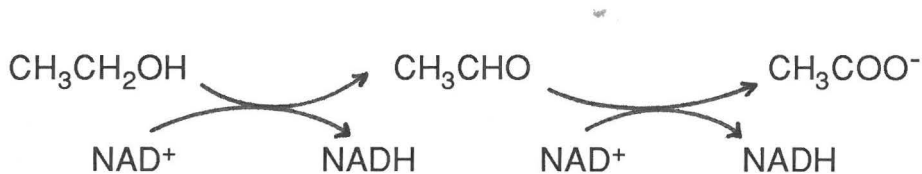
Commentary

Introduction

The scientific publications contained herein constitute the results of nearly twenty years of research into aldehyde dehydrogenase, disulfiram, and related topics. I came to the Department of Chemistry and Biochemistry at Massey University in January 1972 as a Post-Doctoral Fellow after having completed an M.A. (First Class Honours) in Natural Science (Chemistry) and a D. Phil. (involving enzyme kinetics and enzyme modification), both at Oxford. Since the years of my doctorate, I hope I have become reasonably proficient in certain biochemical techniques necessary for the study of enzymology (isolation, purification, chemical modification, kinetics, etc.), but I retain a strong interest in chemistry. Organic chemistry was my favourite subject at school and as an undergraduate, and is still my major concern as a University lecturer. Someone once said that "any competent organic chemist can do biochemistry in his spare time". That may be an exaggeration, but there is no doubt that a sure foundation in organic chemistry is extremely useful for biochemical research. It is my belief that the papers presented here illustrate several cases where a sound knowledge and an application of chemistry have corrected mistakes or clarified difficulties in my particular area of biochemical research.

Aldehyde dehydrogenase

Since the early 1970s, and originally at the instigation of Professor R.D. Batt, this Department has had a major interest in various aspects of alcohol metabolism. Ethanol is metabolised in the liver by this route:



The second oxidation is catalysed by aldehyde dehydrogenase. This enzyme has a broad substrate specificity and also appears to be important in removing any other toxic aldehyde that is ingested or produced endogenously. The fundamental aim of many of the accompanying papers is to characterise aldehyde dehydrogenase fully, to understand exactly how it works, to identify the amino acid residues responsible for its catalytic activity, and to elucidate the details of the step-by-step chemical mechanism by which acetaldehyde is converted to acetate.

Disulfiram

Disulfiram, $(\text{C}_2\text{H}_5)_2\text{N-CS-SS-CS-N}(\text{C}_2\text{H}_5)_2$, was discovered in 1948 to cause an unpleasant reaction to alcohol if it (disulfiram) is ingested beforehand. Since then it has been widely used throughout the world to help alcoholics remain abstinent. The prime cause of the symptoms is that disulfiram inactivates aldehyde dehydrogenase so that acetaldehyde produced in the metabolism of ethanol cannot be quickly disposed of. The interaction between the enzyme and disulfiram in vitro has been greatly illuminated by the work presented here. However, exactly how aldehyde dehydrogenase is inactivated physiologically is still a matter of debate and this problem is addressed by many of the accompanying papers.

Other anti-alcohol compounds

There are many substances that cause a disulfiram-like reaction to alcohol, but apart from disulfiram itself only cyanamide has been used in alcoholism therapy (see Paper no. 30). Certain

cephalosporin antibiotics constitute an important class of compounds that cause an 'accidental' anti-alcohol reaction; my work has elucidated the chemistry behind this phenomenon.