Preparative-scale Reductions of Cyclic Ketone and Aldehyde Substrates of Horse Liver Alcohol Dehydrogenase with *in situ* Sodium Dithionite Recycling of Catalytic Amounts of NAD

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Summary During preparative-scale reductions of cyclic ketone and aldehyde substrates of horse liver alcohol dehydrogenase, up to 105-fold recycling of catalytic amounts of coenzyme has been achieved by *in situ* regeneration of NADH with sodium dithionite; the results indicate that >70% yields of the corresponding alcohols will be obtained provided the rate of reduction of a carbonyl compound is not less than 0·01 times that of cyclohexanone.

EXCEPT in microbiological transformations,[1] the potentials of alcohol dehydrogenases for effecting stereospecific and/or selective oxidation–reductions have been exploited to a limited extent in preparative organic chemistry. The fact that the coenzymes required would be prohibitively expensive if used in stoicheiometric or greater amounts in large-scale reactions has been recognized by several groups[2–6] and the problem has been overcome in a limited number of cases by recycling catalytic amounts of the coenzyme *via* coupled substrate[4,6] or coupled enzyme[2–4] methods. For simple acyclic substrates, high (10^2—10^3) recycling efficiencies are achievable.[3,6] However, with cyclic substrates of more general interest only 15- to 35-fold coenzyme turnovers have been reported.[2,4] Chemical recycling of the coenzyme appeared to us to be an attractive possibility; we report that with sodium dithionite as the reducing agent, up to 105-fold turnovers of catalytic quantities of NAD†‡ have been achieved during preparative-scale reductions of some aldehyde and ketone substrates of HLAD.

The use of dithionite for the reduction of NAD to NADH is appealing since the reagent is inexpensive and is the only one for which exclusive 1,4-reduction of NAD occurs quantitatively to give NADH possessing full coenzymic activity.[7–9] Results of reductions of cyclohexanone are summarised in Table 1.§ Although the coenzyme recycling capability of the method can undoubtedly be improved, the turnovers already achievable by *in situ* dithionite reduction are preparatively viable. Probably as a result of the progressive inactivation of HLAD by NAD,[10] the most efficient coenzyme recyclings were achieved when the enzyme–coenzyme ratio was highest. The recycling efficiency was also dependent on the dithionite concentration, and a minimum threshold¶ of 0·1M was required to ensure high yields of cyclohexanol. Although aqueous

TABLE 1

Reduction of cyclohexanone with HLAD by use of sodium dithionite-recycled NAD

Cyclohexanol (% yield)	[HLAD]/mg ml^{-1}	[NAD]/mM	Rel. ratio [HLAD] : [NAD]	NAD recycles
81	0·06	0·2	1	14
30	0·06	0·02	10	50
98	0·6	0·2	1	16
96	0·6	0·1	20	32
83	0·6	0·05	40	50
63	0·6	0·02	100	105

Dithionite concentration 0·1M.

TABLE 2

Preparative-scale reductions of representative cyclic ketone and aldehyde substrates of HLAD*

Substrate	Rel. redn. rate	Product (% yield)†
Cyclohexanone	1[a]	Cyclohexanol (81‡; 98§)
Cycloheptanone	0·004[b] (0·4[a])	Cycloheptanol (2‡; 4§)
Cyclo-octanone	0·0005[b]	Cyclo-octanol (<1‡; 2§)
Cyclononanone	Not assessed	Cyclononanol (<1‡; 2§)
Adamantanone	0·006[b]; 0·002[c]	Adamantanol (<1‡; 3§)
(*RS*)-3-Methylcyclohexanone	0·4[a]	(1*S*,3*R*)-3-Methylcyclohexanol (46)¶
Benzaldehyde	11[a]	Benzyl alcohol (90)‡
Cinnamaldehyde	70[a]	Cinnamyl alcohol (84‡; 19§)
Furfuraldehyde	47[a]	Furfuryl alcohol (3‡; 76§)

* 0·1M-Dithionite and 0·2mM-NAD. † From quantitative g.l.c. analysis. ‡[HLAD] 0·06 mg ml^{-1}. § [HLAD] 0·6 mg ml^{-1}. ¶ Unreduced (3*R*)-3-methylcyclohexanone (46%) was also recovered.[14] [a] H. Sund and H. Theorell, in ref. 15a, pp. 38—41. [b] J. B. Jones and W. Higgins, unpublished results. [c] H. J. Ringold, T. Bellas, and A. Clark, *Biochem. Biophys. Res. Comm.*, 1967, **27**, 361.

† Abbreviations: NAD, nicotinamide adenine dinucleotide (oxidized form); NADH, nicotinamide adenine dinucleotide (reduced form); HLAD, horse liver alcohol dehydrogenase; YAD, yeast alcohol dehydrogenase.

‡ NADH itself was not used since NAD is both cheaper and more stable.

§ A similar distribution of coenzyme turnovers was observed with the acyclic substrate butyraldehyde.

¶ A similar dithionite-threshold effect has been observed in studies involving YAD-catalysed reductions of acetaldehyde.[11]

dithionite solutions are unstable[12] no problems were encountered in the buffered reaction media employed.

Table 2 summarises results for other cyclic ketone and aldehyde substrates of HLAD. In these reactions the largest volumes surveyed (120 ml) represent 50—60 mg of substrate. The possibility that the substrate reductions were being effected by dithionite alone was eliminated by appropriate control experiments. In 1·25M-dithionite, however, reduction of cyclohexanone did occur to the extent of 10%. A comparison of the relative rates of reduction of the various substrates with the yields obtained of the corresponding alcohols in these and previous[2,14] studies indicates that preparative-scale reductions should be practicable for all carbonyl substrates reduced at a rate greater than *ca.* 0·01 times that of cyclohexanone.** Evidence that the dithionite-recycling process was not interfering with the stereospecificity of the enzyme was provided by the kinetic resolution achieved during the selective reduction of (*RS*)-3-methylcyclohexanone.[14]

Although the presence of dithionite can prove beneficial to some enzymes,†† its effect on HLAD is deleterious, presumably owing to reaction with thiol groups,[15] and the yields of alcohol are markedly decreased when HLAD is pre-incubated with 0·1M-dithionite for 2 h. However, prior addition of NAD and substrate together provides effective protection against such inactivation. The preparative applicabilities of this and other recycling methods to more complex ketones and to other alcohol dehydrogenases are being evaluated.

Support by the National Research Council of Canada is acknowledged.

(*Received*, 12*th May* 1972; *Com.* 817.)

** The reasons for the inconsistent cinnamaldehyde and furfural reduction results are not understood. Some $\alpha\beta$-unsaturated carbonyl compounds are reactive towards dithionite[13] and this aspect is being investigated further.

†† The effect of dithionite on enzymes in general is unpredictable.[11,15]

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