Dioldehydrase: New Observations Concerning the Effect of Oxygen, Nitrous Oxide and Carbon Monoxide

G. N. Schrauzer, Jane A. Seck, and R. J. Holland

Department of Chemistry, The University of California at San Diego, Revelle College, La Jolla, California, U.S.A. 92037

Z. Naturforsch. 28c, 1—3 [1973]; received November 6, 1972

Dioldehydrase, coenzyme B₁₂, oxygen, nitrous oxide, carbon monoxide

Nitrous oxide, N₂O, specifically oxidizes nucleophilic Co(I) derivatives of vitamin B₁₂ and related model compounds and was used successfully to detect the presence of the Co(I) nucleophile derived from coenzyme B₁₂ in functional dioldehydrase holoenzyme. A similar effect of N₂O was also demonstrated in a catalytic model system, in which a cobaloxime(I) nucleophile functions as the catalyst for the conversion of 2-bromoethanol to acetaldehyde. The kinetic behavior of the model system and dioldehydrase holoenzyme are virtually identical, suggesting that N₂O in both cases specifically oxidizes the Co(I)-derivatives present. However, other authors have since studied the effect of N₂O on dioldehydrase and were unable to obtain similarly high degrees of inactivation. Finlay, et al., for example, reportedly observed 15% diminution of dioldehydrase activity in contrast to 75—85% inactivation reported by us. The authors of ref. 3 furthermore quote results of Fukui, who observed no inhibition at all with his dioldehydrase preparations. This contradictory evidence has been resolved on the basis of experiments to be reported in this communication. It is suggested that dioldehydrase holoenzyme exists in two modifications, of which only one is N₂O sensitive. We also include parallel observations with O₂, which is known to inactivate the enzyme-coenzyme complex giving rise to hydroxocobalamin. We will furthermore show that the N₂O sensitive modification of dioldehydrase holoenzyme is inactivated by CO.

Our study of the N₂O effect on dioldehydrase indicated striking variations of the degree of N₂O sensitivity with the source and age of the enzyme preparations. Freshly dissolved solutions of apoenzyme assayed immediately usually showed the highest sensitivity to N₂O; the N₂O effect was occasionally absent in aged, but otherwise active enzyme preparations. We subsequently found that dioldehydrase apoenzyme is converted into an N₂O insensitive modification on exposure to air. Typical results and experimental conditions obtained with a commercial preparation of dioldehydrase are described below:

Two vials of dioldehydrase were dissolved in 2.2 ml of Buffer E under complete exclusion of air, using pure argon as the protecting gas. After 30 min of standing at 25°C this dioldehydrase solution was assayed for activity in rubber serum capped reaction tubes of 25 ml capacity, which contained pure Ar, N₂O, CO, and O₂, respectively, all at 1 atm of partial pressure. The reaction tubes were filled successively with 0.2 ml of 0.2 F pH 8.0 potassium phosphate buffer solution, 0.4 ml of boiled, deionized HO₂, 0.2 ml of an aqueous solution containing 16 μg of coenzyme B₁₂, and 0.1 ml of apoenzyme solution. All operations involving the coenzyme were performed in dimmed light; the reaction tubes were covered with aluminium foil to eliminate light-inactivation of the coenzyme. Aliquots of the reaction solutions (0.2 ml) were withdrawn after 10, 20, 30, and 40 min of incubation at 37°C and assayed for propionaldehyde by the standard spectrophotometric procedure. The results of a typical experiment are shown graphically in Fig. 1 A. With this enzyme preparation the observed inactivation by N₂O, CO, and O₂ was 38, 48, and 56% relative to the run under argon. Storage of an aliquot of the apoenzyme solution under...
arg on for 24 hours at 5 °C caused a 4 % loss of di­
oldehydrase activity and diminished the inhibition
by N₂O, CO, and O₂ to 20, 25, and 52.2 %, respect­
ively. Storage of the same enzyme solution under air
for 24 hours at 5 °C caused virtual disappearance
of N₂O and CO inactivation, while causing only a 7 %
loss of total dioldehydrase activity (Fig. 1 B). The
degree of inactivation by the three gases varied with
the enzyme preparation, but oxygen was invariably
more effective than CO and N₂O.

The effect of air on dioldehydrase apoenzyme sug­
gests the presence of sulfhydryl groups in the vicinity
of the coenzyme binding site. This interpretation is in
accord with independent conclusions reached by To­
raya et al.⁹; dioldehydrase activity has previously
also been shown to be diminished in the presence of
SH blocking reagents such as p-mercuribenzoate¹⁰.
Addition of this reagent at the beginning of the re­
action leads to almost complete inhibition. After com­
pletion of the lag period (20 min), no inhibition is
observed.¹¹ These findings suggest that SH groups are
involved in coenzyme binding and sensitive to the
blocking reagent. Dioldehydrase apoenzyme stored
under argon and dissolved anaerobically reacts with
Ellman's Reagent¹², indicating the presence of free
sulfhydryl groups. Exposure of the apoenzyme to air
indicated a 37 % diminution of the amount of SH
groups relative to the argon-stored apoenzyme. Since
dioldehydrase is normally isolated and purified with­
out consideration of its oxygen sensitivity, it is now
understood why the degree of inactivation by the
gases employed is subject to considerable variation
and dependent on the time and mode of storage of
the apoenzyme. Most of the dioldehydrase prepara­
tions accessible to us showed the presence of some of
the reduced form. However, since subsequent purifi­
cation of the apoenzyme is usually performed aero­
bically, it is clear that this will cause further diminu­
tion of the amount of reduced form present; this is
why Finlay et al.⁹ only observed 15 % inactivation
of holoenzyme by N₂O, and Fukuı none at all. At­
ttempts to reduce the oxidized apoenzyme back to the
reduced form have been partially successful using ex­
cess dihydrolipoic acid as the reductant in the pre­
ence of traces of selenide or aquocobinamide as cata­
lysts, followed by anaerobic dialysis to remove the
thiol and catalysts for disulfide reduction. The partial
reduction of apoenzyme was indicated by the increase
of oxygen sensitivity from 18 to 37 %, and the reap­
pearance of 9 and 7 % of the CO and N₂O inactiva­
tion, respectively.¹³

Oxygen is presumably the strongest inactivator be­
cause of its small molecular size and its ability to oxi­
dize enzyme-bound coenzyme to the Co(III) state.
Nitrous oxide inactivates to a lesser degree presum­
bly because of its larger size and because it can only
react with the Co(I) nucleophile, oxidizing it to
Co(II) rather than Co(III).¹⁴ The inactivation by both
O₂ and N₂O is irreversible and must occur during the
first 10 min of incubation, since the rates of propio­
naldehyde formation after inactivation are essentially
constant. The inactivating effect of CO was invariab­
ly found to be greater than that of N₂O. It is postu­
lated that CO forms a complex with enzyme-bound
corrin Co(I) nucleophile; Co(I) derivatives of cobal­
oximes form CO adducts in solution¹⁵. Although an
analogous adduct with vitamin B₁₂ has not yet been
observed in solution, it could form in the enzyme.
Attempts to reactivate CO-inhibited holoenzyme, e.
.g., by light-irradiation, thus far have been unsuccess­
ful.

It appears that the SH groups in the vicinity of the
coenzyme binding site are unessential for catalytic
activity, just as is apparently the case in ribonucleo­
tide reductase from Lactobacillus leichmannii.¹⁶ Oxidation
of the SH groups to disulfide does not lower the
overall activity of the holoenzyme significantly
but appears to protect the active site against inactiva­
tion by gaseous oxidizing- or blocking reagents by way
of a conformational change of the apoenzyme in the
vicinity of the coenzyme active site.
The inactivation of reduced dioldehydrase holoenzyme by \text{N}_2\text{O} confirms the presence of the Co(I) nucleophile in functional holoenzyme. Since \text{N}_2\text{O} oxidizes Co(I) to Co(II), Co(II)-corrin derivatives are probably not involved in the actual process of enzymatic catalysis. These observations reaffirm the mechanism of dioldehydrase action proposed by us\textsuperscript{17}, which to date is the only mechanism supported by nonenzymatic model experiments.

This work was supported by Grant GP 28485 X of the National Science Foundation, and a NIH Training Grant (PHS 2-TO1-GM-01065-09) for J. A. Seck.

\textsuperscript{1} Abbreviations: Dioldehydrase is di-1,2-propanediol hydrolase, E. C. 4. 2. 1. 28; coenzyme B\textsubscript{12} is \textalpha{}-(5,6-dimethylbenzimidazolyl)-Co-5'deoxyadenosylcobamide. Cobaloximes are derivatives of bis(dimethylglyoximato)cobalt.

\textsuperscript{2} G. N. Schrauzer, R. J. Holland, and J. A. Seck, J. Amer. Chem. Soc. 93, 1503 [1971].


\textsuperscript{5} Obtained from Pierrel S.p.A., Milan, Italy.

\textsuperscript{6} Buffer E is a 0.01 F solution of K\textsubscript{2}HPO\textsubscript{4} containing 20 ml of di-1,2-propanediol per L. The addition of bovine serum albumin to Buffer E for stabilization of dioldehydrase is occasionally recommended, but none was added in the exerpriments reported here.


\textsuperscript{8} The \text{N}_2\text{O} was a commercial product (Linde) of 99\% purity containing traces of water and air as only contaminants. Traces of oxygen from both \text{N}_2\text{O} and CO were removed by passage of the gases through alkaline pyrogallol solution.

\textsuperscript{9} T. Toraya, M. Kondo, Y. Isemura, and S. Fukui, Biochemistry 11, 2599 [1972].


\textsuperscript{11} All dioldehydrase assays in ref. 10 were performed aerobically.

\textsuperscript{12} G. L. Ellman, Arch. Biochem. Biophysics 82, 70 [1959].

\textsuperscript{13} Exposure of this partly reduced apoenzyme to air again diminishes the \text{O}_2 inactivation and causes disappearance of the \text{N}_2\text{O} and CO effects. Addition of 0.1 ml of 0.1 F sodium arsenite solution partially protects the apoenzyme against reoxidation under the experimental conditions outlined in the text.


\textsuperscript{17} G. N. Schrauzer and J. W. Sibert, J. Amer. Chem. Soc. 92, 1022 [1970].