The stereospecificity of the oxidative reaction in the alcohol-dehydrogenase-catalyzed dismutation of aldehydes

Highly purified crystalline horse-liver alcohol dehydrogenase catalyzes the dismutation of formaldehyde and other aldehydes to the corresponding acid and alcohol[1, 2]. Evidence has been presented that alcohol dehydrogenase is the only enzyme required for this dismutation[2]. The following reaction sequence is involved:

$$\begin{array}{lll} CH_2O + DPN^+ + H_2O & \longrightarrow HCOOH + DPNH + H^+ & (1) \\ CH_2O + DPNH + H^+ & \longrightarrow CH_3OH + DPN^+ & (2) \\ \hline 2CH_2O + H_2O & \longrightarrow CH_3OH + HCOOH & (3) \end{array}$$

The exclusive utilization of only one side of the nicotinamide ring of DPN$^+$ by alcohol dehydrogenase during the oxidation of alcohols and reduction of aldehydes has been well established[3]. It therefore appeared of interest to determine whether reaction (1), the oxidation of an aldehyde, shows the same stereospecificity as reaction (2). If reactions (1) and (2) utilize the same side of the nicotinamide ring, the dismutation of an aldehyde containing deuterium on C-1 will not introduce deuterium into DPN$^+$, since the deuterium which the DPN$^+$ acquires in reaction (1) will be removed in reaction (2). On the other hand, if the two reactions show opposite stereospecificities, then the dismutation of the C-1 deutero-aldehyde will result in introduction of deuterium into DPN$^+$.

We carried out the dismutation of CH_3CDO in the presence of DPN$^+$ and alcohol dehydrogenase. CH_3CDO was synthesized according to published procedures[4]. Alcohol dehydrogenase was purchased from Worthington Biochemical Company. The dismutation reaction was carried out as described previously[2]. The following concentrations of reactants were used: DPN$^+$, $7.9 \cdot 10^{-4} M$; enzyme, $6 \cdot 10^{-7} M$; acetaldehyde, $7.2 \cdot 10^{-3} M$. The total volume of the reaction mixture was 20 ml. In order to maintain a nearly constant reaction rate an additional 80 μmoles of acetaldehyde were added after 25 min. Under these conditions, the rate of acid formation was maintained between 1.1 and 1.5 μmoles/min. The reaction was allowed to proceed for 60 min; 78.4 μmoles of acid were produced during this time. The nicotinamide portion of the DPN$^+$ was then isolated[5] and its deuterium content determined. No deuterium above the naturally occurring amount could be detected. These results, therefore, indicated that both the alcohol-dehydrogenase-catalyzed oxidation (1) and reduction (2) utilize the same side of the nicotinamide ring of DPN$^+$.

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Abbreviation: DPN$^+$, DPNH, oxidized and reduced diphosphopyridine nucleotide.

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