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Received March 31st, 1970

*Biochim. Biophys. Acta*, 212 (1970) 501–503

BBA 63476

## Stereochemistry of glyoxylate oxidation by NAD and mammalian lactate dehydrogenase

The NAD-dependent oxidation and reduction of glyoxylate to oxalate and glycolate, respectively, are catalyzed by L-lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27). It has been proposed that both reactions occur in the same active site(s) and involve common functional catalytic groups of the enzyme[1]. If the same active sites, including binding and catalytic groups, are utilized for both reactions, the transfer of hydride ion to and from the NAD coenzyme should take place on the same side of the pyridine ring in glyoxylate oxidation and in glyoxylate reduction. In reduction reactions involving L-lactate dehydrogenase and NADH, the hydride ion is transferred from the A side[2] of the dihydropyridine ring[3,4]. In the present investigation it is shown that glyoxylate oxidation by L-lactate dehydrogenase involves transfer of a hydride ion from glyoxylate to the A side of the 4 position of the pyridine ring of $NAD^+$.

Glyoxylate*, containing $^3H$ on the aldehyde carbon, was prepared in $^3H_2O$ (5 mC) from oxalic acid and magnesium[5], and the glyoxylate was separated from oxalate by ion-exchange chromatography[1]. The specific activity of the [$^3$H]glyoxylate was 7000 disint./min per $\mu$mole. [$^3$H]NADH was obtained by oxidation of 40 $\mu$moles of [$^3$H]glyoxylate with 40 $\mu$moles of $NAD^+$ by 2.5 mg of pig heart lactate dehydrogenase at pH 11 (ref. 1). Oxalate was precipitated with $BaCl_2$, and barium [$^3$H]NADH was then precipitated with alcohol[6]. The specific activity of the [$^3$H]NADH was 5700 disint./min per $\mu$mole.

Reaction mixtures containing glycolate and $NAD^+$, formed from glyoxylate and [$^3$H]NADH, were streaked onto Whatman No. 3 MM chromatography paper. The paper was developed by ascending chromatography in ethanol–conc. $NH_4OH$–water (80:5:15, by vol.). The $R_F$ for glycolate, determined by migration of [$^{14}$C]glycolate along one edge of the paper, was 0.44, and that for $NAD^+$, determined by ultraviolet visualization, was 0.04. The appropriate portions of the paper were cut out and eluted

* Glyoxylate is $O^1HCCOO^-$ and tritiated glyoxylate is $O^3HCCOO^-$.

with water. Reaction mixtures containing glutathione and $NAD^+$, formed from oxidized glutathione and [$^3$H]NADH, were chromatographed on a column (1.4 cm × 4.5 cm) of AG-50W-X2 ($H^+$). The $NAD^+$ was eluted with water in the 30–70-ml fraction, and the glutathione was subsequently eluted with 12 M formic acid in the 40–100-ml fraction.

Glyoxylate was determined by reaction with resorcinol[7], glycolate at 570 nm after reaction with 4,5-dihydroxynaphthalene-2,7-disulfonic acid[8], NADH by absorbance at 340 nm[9], and $NAD^+$ by absorbance at 260 nm[10]. $^3$H was measured by liquid scintillation, and the disint./min were determined by the channels ratio method.

By oxidizing [$^3$H]NADH in the presence of two enzymes, one specific for the hydrogen on the A side and the other specific for the hydrogen on the B side of the dihydropyridine ring of NADH, the question of the location of the $^3$H in [$^3$H]NADH, produced by reduction of $NAD^+$ through oxidation of [$^3$H]glyoxylate by pig heart L-lactate dehydrogenase, was answered unequivocally (Table I). Mammalian L-lactate dehydrogenases are known to be specific for the hydrogen on the A side, while yeast

TABLE I

STEREOSPECIFICITY OF CATALYTIC REDUCTION OF GLYOXYLATE AND OF OXIDIZED GLUTATHIONE WITH [$^3$H]NADH

Reaction I, reduction of glyoxylate by L-lactate dehydrogenase. Glyoxylate (10 μmoles), barium [$^3$H]NADH (3.3 μmoles) and pig heart lactate dehydrogenase (10 μg) were included in 1 ml at 25°, pH 6. The pH was maintained between 6 and 6.5 by addition of HCl. After 15 min, no further change in pH or absorbance at 340 nm occurred, and only 2% of the initial amount of [$^3$H]-NADH remained. Glycolate and $NAD^+$ were separated by paper chromatography. Reaction II, reduction of oxidized glutathione by glutathione reductase. GSSG (10 μmoles), barium [$^3$H]-NADH (3.2 μmoles) and yeast glutathione reductase (125 μg) were included in 1 ml at 25°, pH 7.4. The pH was maintained between 7.2 and 7.6 by addition of HCl. After 100 min, only 8% of the initial amount of [$^3$H]NADH remained, and glutathione and $NAD^+$ were separated by ion-exchange chromatography.

| *Reaction* | *Product* | *Specific activity (disint./min per μmole)* |
|---|---|---|
| I | $NAD^+$ | 0 |
| | Glycolate | 5250 |
| II | $NAD^+$ | 3550 |
| | Glutathione | 12 |

glutathione reductase is specific for the hydrogen on the B side[3,4]. When the [$^3$H]-NADH was oxidized with glyoxylate and pig heart lactate dehydrogenase, the $^3$H was transferred completely to the glycolate product, and the resultant $NAD^+$ was entirely free of radioactivity. This result indicated that the $^3$H of the [$^3$H]NADH was on the A side of the 4 position of the dihydropyridine ring. Confirmation of this finding was provided by reducing oxidized glutathione with the [$^3$H]NADH in the presence of yeast glutathione reductase. In this case, the $^3$H was in the $NAD^+$ product, and the reduced glutathione was essentially nonradioactive.

Tentative mechanisms for the NAD-dependent oxidation and reduction of glyoxylate by mammalian L-lactate dehydrogenase were based on the assumption

that the same active site was utilized for catalysis of both reactions[1]. Implicit in this hypothesis is the notion that $NAD^+$ and NADH are bound by the same protein functional groups so that the orientations of the pyridine and dihydropyridine rings are the same with respect to the configuration of the active site. If this hypothesis be true, then hydride ion transfer from glyoxylate to $NAD^+$ in glyoxylate oxidation should proceed to the same side (A) of the 4 position of the pyridine ring as the side from which a hydride ion is given when glyoxylate is reduced with NADH. This result was found in the oxidation of [$^3$H]glyoxylate with $NAD^+$ by pig heart lactate dehydrogenase.

The technical assistance of Caroline Brown in part of this work is gratefully appreciated. This investigation was supported by Grants AM-12440 and FR-05498 from The United States Public Health Service, and by the Stephen Carlton Clark Research Fund of The Mary Imogene Bassett Hospital.

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Received May 19th, 1970

*Biochim. Biophys. Acta*, 212 (1970) 503–505

BBA 63473

## The isolation of arylsulphatase isoenzymes from *Pseudomonas aeruginosa*

The precise nature and specific metabolic function of bacterial arylsulphatase (arylsulphate sulphohydrolase, EC 3.1.6.1) is presently unresolved. Only one such enzyme has been obtained in homogeneous form and many of its properties were found to be distinct from those of mammalian arylsulphatase[1,2]. Other workers have advanced the possibility of multiple forms of this enzyme in *Proteus rettgeri*[3,4] and *Proteus vulgaris*[5] and it has been suggested from studies with cell extracts of *Pseudomonas aeruginosa* that several sulphatases were synthesized depending upon the nature of the sulphur source in the growth medium[6].

The implication of the latter study is confirmed by the present work which describes the first isolation of two highly purified arylsulphatase isoenzymes from a bacterium.