THE STEREOSPECIFICITY OF THE DPNH OXIDASE REACTION

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Previously, Drysdale and Cohn (1956) studied the reaction of DPNH cytochrome c reductase, prepared from pig heart muscle by the method of Mahler et al (1952), and found it to be stereospecific for the β configuration of DPNH. More recently, De Bernard (1957) and Mackler (1961) have prepared DPNH cytochrome c reductases from highly purified preparations of beef heart electron transport particles (ETP) and DPNH oxidase respectively, by modifications of the method of Mahler et al (1952). The present paper describes the results of studies of the α,β-stereospecificity and the effects of deuterium oxide on the rates of reactions of DPNH oxidase and the derivative DPNH dehydrogenase.

DPNH oxidase was isolated as described by Mackler and Green (1956). DPNH dehydrogenase was prepared from the purified preparations of DPNH oxidase by modifications of the method of De Bernard (1957). DPND was prepared in the A form (α-isomer) by the reaction of DPN with yeast alcohol dehydrogenase and deuteroethanol, and in the B form (β-isomer) by the cyanide addition method described by San Pietro (1955). Deuterium oxide was obtained from the Eli Lilly Company. Enzymic assays were performed as described previously by Mackler and Green (1956) and by Mackler (1961).

Table I shows the results of studies of the reactions of DPNH oxidase and DPNH dehydrogenase with forms A and B of DPND. As shown in the Table both of the enzymes react with form A of DPND at a rate equal to that with DPNH. However, when form B of DPND was used in place of DPNH, rates of reaction for DPNH oxidase (oxygen as acceptor) and DPNH dehydrogenase (with 2,6 dichlorindophenol as acceptor) were reduced by 78% and 40% respectively.

TABLE I

Effects of α and β-DPND on enzymic catalysis

Enzyme Preparation	Acceptor	Inhibition (%) with deuterated isomers of DPNH	
		α-DPND	β-DPND
DPNH oxidase	Oxygen	0	78
DPNH dehydrogenase	Oxygen	0	0
	Cytochrome c	0	< 10
	FAD	0	0
	Indophenol	0	40

Thus DPNH oxidase and DPNH dehydrogenase are specific for the β configuration of DPNH. When acceptors other than indophenol were used in the reaction with β-DPND and DPNH dehydrogenase little or no inhibition was found, indicating that the initial reactions of DPNH (or DPND) with the enzyme are not rate limiting for these acceptors.

TABLE II

Effects of deuterium oxide on the enzymic activity of DPNH dehydrogenase

Acceptor	Rate in D_2O (% of rate in H_2O)
Indophenol	100
Cytochrome c	100
Oxygen	28
FAD	30

Table II shows the results of experiments on DPNH dehydrogenase performed with 95% deuterium oxide present in the assay systems instead of water. As demonstrated in the Table no effects on the enzymic rate were observed with indophenol or cytochrome c as acceptors, but a 30% inhibition was found with oxygen or FAD as acceptor in the reaction. The results add further support to the finding that an initial reaction involving the β hydrogen from DPNH is rate limiting in the indophenol catalyzed reaction, but that the terminal reaction between acceptor and hydrogen ions (from the assay medium) is rate limiting in the enzyme catalyzed reactions with oxygen and FAD. The finding that deuterium oxide does not affect the enzymic reaction with cytochrome c is not unexpected, since only the passage of an electron is involved in the reduction of ferricytochrome c to ferrocytochrome c.

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