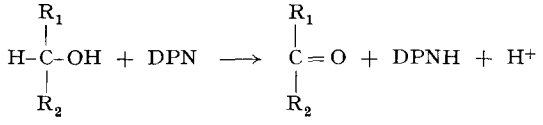
Reaction of thiol compounds with pyridine nucleotides§

It has previously been reported that a complex is formed between DPN and hydroxylamine in the presence of horse liver alcohol dehydrogenase[1]. A similar complex has also been observed between hydroxylamine, the pyridine-3-aldehyde analogue of DPN and yeast alcohol dehydrogenase[2]. Recently, TERAYAMA AND VESTLING[3] have reported a complex between liver lactic dehydrogenase, DPN and sodium sulfide.

We wish to present evidence, indicating that complex formation between pyridine coenzyme, enzyme, and a nucleophilic compound structurally related to the substrate, is a general reaction of all dehydrogenases which carry out the following type of reaction:



We have found that this type of dehydrogenase will interact with thiol derivatives and pyridine nucleotides.

All thiols examined, including sodium sulfide, mercaptoacetic acid, mercaptosuccinic acid, thioethanol and *n*-alkyl-mercaptans, react non-enzymically with pyridine nucleotides to form addition products. In some instances where DPN shows little reaction, DPN analogues (*i.e.* the acetylpyridine or pyridine-3-aldehyde analogues of DPN[4]) will form addition complexes. In the presence of a suitable dehydrogenase this reaction between coenzyme and thiol compound becomes

TABLE I

THE ENZYME-COENZYME-SUBSTRATE COMPLEX FOR DIFFERENT DEHYDROGENASES

Spectra were taken in 0.1 *M* phosphate buffer, pH 7.4. The reference cell contained the thiol compound, the experimental cell thiol derivative and enzyme. After this spectrum was taken, the coenzyme was added to both reference cell and experimental cell. The addition-complex spectrum was computed from the difference of the two spectra obtained. A Beckman spectrophotometer, model DK was used throughout.

Dehydrogenase	*Coenzyme or coenzyme analogue*	*Thiol-derivative*	E_{max} *enzymic complex* mμ	E_{max} *non-enzymic complex* mμ
Liver alcohol dehydrogenase	DPN	ethylmercaptan	315	330
Yeast alcohol dehydrogenase	APDPN*	ethylmercaptan	340	355
Beef heart lactic dehydrogenase	DPN	mercaptosuccinic acid	310	330
Skeletal muscle lactic dehydrogenase	DPN	mercaptosuccinic acid	310	330
Liver lactic dehydrogenase	DPN	sodium sulfide	320**	335
Pig heart malic dehydrogenase	Py-3AlDPN*	mercaptosuccinic acid	320	355
Beef liver glutamic acid dehydrogenase	DPN	mercaptoacetic acid	315	330
Rabbit muscle glycerol phosphate dehydrogenase	Py-3AlDPN*	propane-1,2-dithiol	345***	355

* APDPN and Py-3AlDPN stand for the acetylpyridine and pyridine-3-aldehyde analogues of DPN.

** Data taken from TERAYAMA AND VESTLING[3].

*** In contrast with all other complexes reported in this table, the complex on this enzyme forms with a measurable rate, rather than instantaneous.

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much more favorable. For example, in the case of yeast alcohol dehydrogenase the reaction between the acetylpyridine analogue of DPN and ethylmercaptan is approximately a million times more favorable than when the reaction is carried out in the absence of enzyme. It is of interest to note that although DPN shows little reaction with yeast alcohol dehydrogenase and ethylmercaptan, crystalline horse liver alcohol dehydrogenase will readily form a complex with the thiol compound and DPN (see Table I).

The reaction between the enzyme, coenzyme and substrate analogue exhibits specificity. Only those thiol derivatives which show a sufficient structural resemblance to the normal substrate of the various dehydrogenases will react with the pyridine nucleotides. For example, beef heart lactic dehydrogenase will react with mercaptoacetic or mercaptosuccinic acids, but not with 2-mercaptoethanol. Similar specificity is shown by skeletal muscle lactic dehydrogenase. The alcohol dehydrogenases will only form complexes with the *n*-alkyl mercaptans. Malic dehydrogenase will form a complex with mercaptosuccinic acid but not with ethylmercaptan. This enzyme appears to give a far more favorable complex with pyridine-3-aldehyde analogue of DPN than with DPN. Crystalline beef liver glutamic dehydrogenase forms a complex with DPN and mercapto-acetic acid, but not with mercaptosuccinic acid. Crystalline rabbit muscle glycerophosphate dehydrogenase shows a complex with propane-1,2-dithiol but not with thioethanol. Again the complex with the pyridine-3-aldehyde analogue appears to be far more favorable than with DPN.

The chemical reaction between hydroxylamine and DPN has a maximum at 315 mμ[5]; in the presence of the horse liver alcohol dehydrogenase the maximum is shifted to 300 mμ[1]. In a similar manner, the absorption maxima of the enzymically-bound thiol complexes are shifted toward shorter wavelengths (Table I). In all cases, where an enzymic reaction has been observed, a non-enzymic complex has also been detected.

The results of this work strongly support the previously proposed hypothesis that an addition reaction of substrate to coenzyme may be the first step in the reaction catalysed by this class of dehydrogenase[5]. Details of the kinetic implications and properties of the thiol-pyridine nucleotide complexes will be published shortly.

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The enzymic oxidation of reduced vitamin K_3 (menadione)

Although enzymes catalysing the reduction of vitamin K_3 (menadione)[1] or of vitamin K_1[2] by reduced diphosphopyridine nucleotide (DPNH) are known, there are no reports in the literature that the resultant hydroquinones can be re-oxidized by mitochondrial preparations. Both hydroquinones are, in fact, rapidly auto-oxidizable by air at neutral pH, but at pH 6.24, in the presence of ethylenediaminetetraacetate (EDTA), the auto-oxidation of the hydroquinone of menadione (K_3H_2) is very slow. It has now been shown, by working at this pH, that sarcosomal (mitochondrial) fragments prepared from horse heart (Keilin and Hartree heart-muscle preparation) actively catalyse the oxidation of K_3H_2 in the absence of added cytochrome *c*.

The special interest of this reaction lies in the fact that, whereas a large number of substances (such as ascorbic acid, benzohydroquinone) can be oxidized by this preparation in the presence of added cytochrome *c*[3], only succinate, DPNH and *p*-phenylenediamine are oxidized in the absence of the added cytochrome. The oxidation of K_3H_2 resembles that of succinate and DPNH rather than that of *p*-phenylenediamine, since it is completely inhibited by antimycin A.

The K_3H_2 oxidase activity was measured spectrophotometrically by following the increase of the optical density at 262 mμ, as shown in Fig. 1. After measuring the rate of the auto-oxidation, the enzymic reaction was started by adding a suitably diluted heart-muscle preparation to each cuvette. After the completion of the reaction, the spectrum was identical with that of menadione, with bands at 250, 262–263 and 340 mμ. The course of the oxidation shows first order kinetics, and the activity of the enzyme was expressed in terms of the first order velocity constant, k'. Doubling the initial concentration of the K_3H_2 did not affect k'.

Since first order kinetics were obtained with K_3H_2, and zero order kinetics are found with comparable concentrations of DPNH[5], it is not possible to compare the specific activities of the