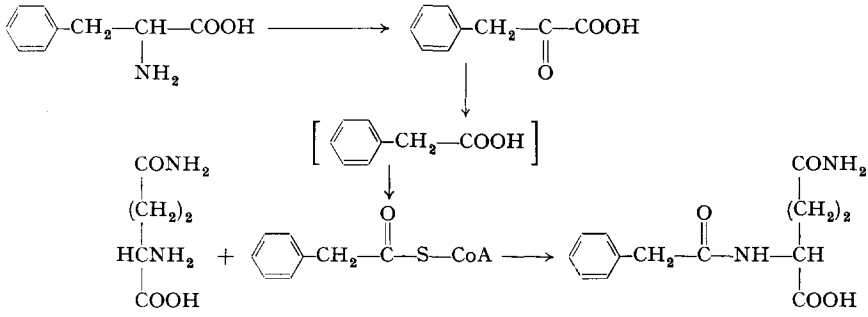
Human liver and kidney were fractionated to yield preparations of the acylating activity that were 40- to 100-fold more active per milligram of protein than the original homogenates. A typical experiment carried out with the purified liver enzyme is described in Fig. 1. When chromatography was carried out with four other solvents, similar results were obtained, and within experimental error, the same amount of ^{14}C-PAG was recovered. In all five solvent systems, the R_F values of ^{14}C-PAG agreed with those of an authentic sample of PAG prepared by organic synthesis from L-glutamine. Elution of the ^{14}C-PAG from the paper strips, followed by acid hydrolysis, yielded glutamic acid as the only radioactive product.

The present results indicate that phenylacetyl-coenzyme A is an intermediate involved in PAG synthesis. Phenylacetyl-coenzyme A may arise in the course of the oxidative-decarboxylation of phenylpyruvate formed from phenylalanine by transamination, or by the activation of exogenous phenylacetate. These reactions may be represented as follows:



Studies on the specificity and other properties of the purified acylating enzyme system obtained from liver and kidney and of the activating system prepared from human liver mitochondria are in progress.

This investigation was supported in part by research grants from the U.S. Public Health Service and the National Science Foundation. The authors thank Dr. CHARLES G. CHILD for his generous assistance in providing biopsy samples and Dr. G. J. GHERARDI for his cooperation in providing the autopsy material.

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Received March 30th, 1957

Specific protection of the thiol groups of aldehyde dehydrogenases by pyridine-nucleotide coenzymes*

The potassium-activated yeast aldehyde dehydrogenase (BLACK[1]), the TPN-linked yeast aldehyde dehydrogenase (SEEGMILLER[2]), and the aldehyde dehydrogenase from liver (RACKER[3]), are inhibited by the following sulfhydryl reagents: a trivalent arsenical compound (3-amino-4-hydroxyphenylarsenoxide or *mapharside*), N-ethylmaleimide, *o*-iodosobenzoate, *p*-chloromercuribenzoate and iodoacetate. The yeast TPN-specific enzyme is comparatively the most sensitive to *p*-chloromercuribenzoate, *o*-iodosobenzoate, N-ethylmaleimide, and iodoacetate, whereas the yeast potassium-activated dehydrogenase is the most sensitive to *mapharside*. These findings allow the inclusion of yeast TPN-specific dehydrogenase in the group of SH enzymes and extend the already existing evidence of the essential role of thiols in the aldehyde dehydrogenases described by BLACK[1] and RACKER[3].

* DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

In some DPN-linked dehydrogenases like glyceraldehyde-3-phosphate dehydrogenases and alcohol dehydrogenases, the role of thiols has been the subject of extensive studies. The sulfhydryls are in a functional relationship to the coenzyme as shown by (a) the effect of SH reagents on the spectroscopic properties of the enzyme-coenzyme compound (RACKER AND KRIMSKY[4], VELICK[5], THEORELL AND BONNICHSEN[6] and KAPLAN AND CIOTTI[7]) and (b) the protection given by the coenzyme against thiol reagents (RAPKINE *et al.*[8], BARRON AND LEVINE[9]). Since a similar role of thiols seemed possible in aldehyde dehydrogenases, the protection of these groups by the coenzyme was investigated. SEEGMILLER's yeast enzyme and the liver aldehyde dehydrogenase reduce specifically TPN and DPN respectively[2,3], whereas the potassium-activated yeast dehydrogenase reduces both DPN and TPN, although the reaction with the first is ten times faster than with the latter[1].

TABLE I

EFFECT OF PYRIDINE-NUCLEOTIDE COENZYMES ON THE INHIBITION OF ALDEHYDE DEHYDROGENASES BY THIOL REAGENTS

Enzymes dissolved in 0.5 ml 0.015 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.0, were treated with additions as shown for 30 sec (expt. A, B, D and E), 1 min (expt. C, F and I), 3 min (expt. H and J) or 5 min (expt. G). 0.05–0.2 ml of these extracts (according to the experiment) were diluted to 3.0 ml with the enzyme assay mixture and the enzyme activity measured.

Experiment	*Additions*	*Enzyme*	
		Inhibition (%)	*Protection (%)*
	Potassium-activated yeast aldehyde dehydrogenase		
A	0.064 m*M mapharside*	72.4	—
A	1.8 m*M* DPN + 0.064 m*M mapharside*	39.8	46.3
A	1.8 m*M* DPNH + 0.064 m*M mapharside*	29.7	59.0
B	0.032 m*M mapharside*	97.3	—
B	1.5 m*M* TPN + 0.032 m*M mapharside*	85.2	12.4
C	0.048 m*M* N-ethylmaleimide	78.6	—
C	1.3 m*M* DPN + 0.048 m*M* N-ethylmaleimide	44.3	43.6
C	1.3 m*M* DPNH + 0.048 m*M* N-ethylmaleimide	47.3	39.8
D	0.06 m*M o*-iodosobenzoate	75.2	—
D	1.3 m*M* DPN + 0.06 m*M o*-iodosobenzoate	27.0	63.8
E	0.02 m*M o*-iodosobenzoate	83.0	—
E	1.5 m*M* TPN + 0.02 m*M o*-iodosobenzoate	36.0	56.7
	TPN-specific yeast aldehyde dehydrogenase		
F	0.02 m*M* N-ethylmaleimide	98.6	—
F	1.5 m*M* TPN + 0.02 m*M* N-ethylmaleimide	22.3	77.5
F	2.7 m*M* DPN + 0.02 m*M* N-ethylmaleimide	98.1	0.9
G	0.004 *M* iodoacetate	52.5	—
G	0.7 m*M* TPN + 0.004 *M* iodoacetate	21.4	59.0
G	2.7 m*M* DPN + 0.004 *M* iodoacetate	54.4	—3.6
	Liver aldehyde dehydrogenase		
H	0.16 m*M mapharside*	72.0	—
H	2.7 m*M* DPN + 0.16 m*M mapharside*	35.2	51.0
I	0.2 m*M o*-iodosobenzoate	72.0	—
I	0.3 m*M* DPN + 0.2 m*M o*-iodosobenzoate	—16.7	123.0
I	0.3 m*M* TPN + 0.2 m*M o*-iodosobenzoate	68.1	5.4
J	0.6 m*M p*-chloromercuribenzoate	84.8	—
J	2.7 m*M* DPN + 0.6 m*M p*-chloromercuribenzoate	64.2	24.3
J	1.5 m*M* TPN + 0.6 m*M p*-chloromercuribenzoate	85.3	—0.6

The experimental procedure was as follows. Neutralized DPN (or TPN), distilled water, and the thiol reagent, were added to the enzyme dissolved in tris(hydroxymethyl)aminomethane buffer, pH 8.0 (final concentration 0.015 M), in a total volume of 0.5 ml. After the required time, measured from the addition of the inhibitor, an aliquot was quickly diluted with the appropriate assay mixture and the dehydrogenase activity was measured as described by BLACK[1], SEEGMILLER[2] or RACKER[3], respectively. Controls treated in the same way except for (a) the thiol reagent, (b) the coenzyme, and (c) thiol reagent and coenzyme, were measured simultaneously. The percentage protection (P) of the dehydrogenase by the coenzyme was calculated by the equation $P = (I_{tr} - I_{c,tr})/I_{tr}$ where I_{tr} is the inhibition (%) of the dehydrogenase by the thiol reagent alone and $I_{c,tr}$ the inhibition (%) of the dehydrogenase treated with the coenzyme and the thiol reagent. All inhibitions were calculated in relation to the corresponding controls.

As shown in Table I, the sensitivity of aldehyde dehydrogenases for SH reagents diminished in the presence of the respective coenzyme. Several points are noteworthy. (1) The state of oxidation of DPN is immaterial for its capacity to protect BLACK's enzyme, since DPNH gave the same or a higher protection than DPN. (2) TPN protects BLACK's enzyme as well as DPN, though the intensity of the protection was comparatively smaller, particularly with *mapharside*. (3) The protection of SEEGMILLER's enzyme by TPN was quite specific, since a similar or a higher concentration of DPN did not affect the enzyme inhibition by N-ethylmaleimide, iodoacetate, or *p*-chloromercuribenzoate. (4) The opposite situation was found with the liver aldehyde dehydrogenase where DPN but not TPN prevented the inhibition by *o*-iodosobenzoate and *p*-chloromercuribenzoate. In the presence of DPN and *o*-iodosobenzoate, the enzyme activity was promoted to a level above that of the control preparation. This unexpected effect was also obtained with DPNH but to a smaller degree.

Similar protection experiments were carried out with acetaldehyde instead of the coenzyme but the results obtained were far less consistent. In fact, acetaldehyde (1) was effective only on BLACK's and RACKER's enzymes; (2) did not prevent the inhibition of these dehydrogenases by SH reagents like iodoacetate and *p*-chloromercuribenzoate, respectively, and (3) even when effective, acetaldehyde was a weaker protector than the pyridine nucleotides, except in the prevention of the inhibition of liver aldehyde dehydrogenase by *mapharside*.

The systematic and specific effect of the coenzyme on the inhibition of aldehyde dehydrogenases by SH reagents shows that the thiols are close to or in the enzyme site that binds the coenzyme. This fact, observed with the three different dehydrogenase preparations, leads to the conclusion that the sulfhydryls must have an important function in connection with the coenzyme, as seems to occur in other DPN-linked dehydrogenases. In addition, in BLACK's and RACKER's enzymes there may be other thiols with a different role, like the binding of the aldehyde group to the protein (*cf.* KOEPPE *et al.*[10]) as will be discussed elsewhere.

A detailed account of this work will be published in another journal. We are grateful to E. R. Squibb and Sons, Argentina, and I.V.A., Industria Vidriera Argentina, for financial assistance, and to "Laboratorios Millet" for a fellowship to C.M.

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Received March 21st, 1957