n-butanol–acide acétique–eau–pyridine (30 : 6 : 24 : 20). Les comportements au cours de l'électrophorèse sur papier à pH 3.5, 6.5 et 8.6 sont également identiques. L'hydrolyse du produit fournit les acides aminés constituants de l'ocytocine des mammifères avec en plus de faibles quantités d'alanine, de sérine et d'arginine. Une purification plus poussée a été obtenue par chromatographie préparative sur papier: l'ocytocine du poulet (180 unités), purifiée sur Amberlite IRC-50 et débarrassée de l'acétate d'ammonium, est chromatographiée dans le solvant *n*-butanol–acide acétique–eau, l'ocytocine du cheval étant utilisée comme témoin. Après révélation de la bande témoin, on élue le matériel correspondant à l'ocytocine, et on hydrolyse par HCl 6 *N*, à 110° pendant 20 h. La composition en acides aminés du produit, établie par chromatographie semi-quantitative sur papier, est la suivante: $(Cys)_2$, Tyr,Ileu,Asp,-Glu,Pro,Leu,Gly, composition identique à celle de l'ocytocine des mammifères.

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Reçu le 24 juillet 1959

Biochim. Biophys. Acta, 38 (1960) 344–345

Studies of DPNH oxidase: on the mechanism of reaction

Previously[1], we have shown that purified DPNH oxidase contains bound diphosphopyridine nucleotide in a fluorescent form, suggesting that it is bound to the enzyme in a reduced state. DPN containing [8-^{14}C]adenine was used to study the mode of binding of the pyridine nucleotide to DPNH oxidase and the relation of the binding to enzymic catalysis. Labeled DPN was obtained from Schwarz Laboratories, Mt. Vernon, New York. Concentrations of DPN and protein were determined as described previously[1]. [^{14}C]DPN was converted to [^{14}C]DPNH by reduction with $Na_2S_2O_4$, excess dithionite being removed by aeration. DPNH oxidase was incubated at room temperature with either [^{14}C]DPNH or [^{14}C]DPN for 20 min and was then washed repeatedly by centrifugation until the wash fluid was free of ^{14}C activity. The washed DPNH oxidase was analysed for total pyridine nucleotide and for ^{14}C-labeled pyridine nucleotide. Table I shows the exchange of [^{14}C]DPN and [^{14}C]-DPNH, at varying concentrations of labeled pyridine nucleotide, with the pyridine nucleotide originally bound to the DPNH oxidase. Only in Expt. 6, after incubation

Abbreviations: DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide.

with very high levels of [^{14}C]DPNH, was the final concentration of enzyme-bound pyridine nucleotide higher than that determined before addition of the labeled nucleotide; in all other instances the two values were equal demonstrating no net uptake of pyridine nucleotide. As shown in the Table, [^{14}C]DPN and [^{14}C]DPNH both exchange with the enzyme-bound pyridine nucleotide to the same degree, and the extent of the exchange is directly dependent on the amount of labeled pyridine nucleotide added. Subsequent assays with alcohol dehydrogenase showed that there was no disappearance of pyridine nucleotide during the course of these experiments since added DPNH was quantitatively recovered as DPN after its reaction with DPNH oxidase. It would therefore appear that the exchange reaction is not dependent on enzymic activity. The non-enzymic nature of the exchange is more directly shown by data from experiments similar to those presented in Table I but performed under anaerobic conditions and in the presence of cyanide. The results were identical to those presented in Table I.

The results presented demonstrate that the enzymic oxidation of DPNH by

TABLE I

EXCHANGE OF ^{14}C-LABELED PYRIDINE NUCLEOTIDE WITH ENZYME-BOUND PYRIDINE NUCLEOTIDE

Expt.	*^{14}C-labeled pyridine nucleotide added**		*Exchange of labeled pyridine nucleotide with bound pyridine nucleotide***	
	[^{14}C]DPNH	*[^{14}C]DPN*	*[^{14}C]DPNH*	*[^{14}C]DPN*
1	3	3	0	0
2	6	6	12	12
3	20	20	22	22
4	190	190	87	87
5	215	—	91	—
6	432	—	100	—

* Expressed as a multiple of the enzyme-bound pyridine nucleotide.
** Expressed as the per cent of enzyme-bound pyridine nucleotide which exchanged with the added ^{14}C-labeled pyridine nucleotide.

DPNH oxidase is not mediated by a direct exchange of substrate (DPNH) with the enzyme-bound pyridine nucleotide. Although there is an exchange of added pyridine nucleotide with that bound to the enzyme, this exchange is dependent on the concentration of pyridine nucleotide added and not on enzymic activity.

This work was supported by grants Nos. H-3898, H-3520, and A-2164 from the National Institutes of Health, U.S. Public Health Service, and by a grant from the Initiative 171 fund, State of Washington. It was performed during the tenure by one of us (B.M.) of an Established Investigatorship of the American Heart Association.

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Received July 29th, 1959