conversion but that its liberation is not a prerequisite for chymotryptic activity.

On the basis of these data, and others previously reported, it now appears possible to identify the amino acid sequence in chymotrypsinogen which is primarily involved in the activation process. The following considerations summarize the pertinent points of evidence: (1) $\pi$-chymotrypsin differs from chymotrypsinogen in possessing an N-terminal isoleucyl-valine sequence,[4,10] both proteins being devoid of a C-terminal group reactive toward carboxypeptidase[4]; (2) the conversion of $\pi$- to $\delta$-chymotrypsin yields a C-terminal leucine group, no new N-terminal group, and the dipeptide seryl-arginine; (3) the action of $\alpha$-chymotrypsin on chymotrypsinogen yields neither enzymatic activity nor the dipeptide,[11] suggesting that seryl-arginine is not a C-terminal sequence in chymotrypsinogen. Omitting from consideration factors arising from the presence of the N-terminal half-cystine group found in chymotrypsinogen[12] it seems most likely that the amino acid sequence involved in activation is leucyl-seryl-arginyl-isoleucyl-valine, and that the arginyl-isoleucine bond is opened in the trypsin-catalyzed formation of $\pi$-chymotrypsin. The subsequent, chymotrypsin-catalyzed[4] conversion of $\pi$- to $\delta$-chymotrypsin involves the hydrolysis of the leucyl-serine bond, giving rise to the dipeptide, a C-terminal leucine group and an N-terminal isoleucyl-valine sequence. It is worthy of note that, as in the activation of trypsinogen,[13] the splitting of a single bond suffices to produce enzymatic activity and that all of these active enzymes have the same N-terminal dipeptide sequence.[14] The splitting of the leucyl-serine bond of $\pi$-chymotrypsin is without effect on the specific esterase activity of the activation mixture. Details of this work will be published elsewhere.

(10) M. Rovery and P. Desnuelle, *Biochim. et. Biophys. Acta*, **13**, 300 (1954); M. Rovery, M. Toilroux and P. Desnuelle, *ibid.*, **14**, 145 (1954).

(11) W. J. Dreyer, unpublished experiments.

(12) F. R. Bettelheim, *J. Biol. Chem.*, **212**, 235 (1955).

(13) E. W. Davie and H. Neurath, *J. Biol. Chem.*, in press.

(14) P. Desnuelle and C. Fabre, *Bull. Soc. Chim. Biol.*, **36**, 181 (1954).

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WASHINGTON WILLIAM J. DREYER
SEATTLE 5, WASHINGTON HANS NEURATH

RECEIVED DECEMBER 4, 1954

## ISOLATION OF A DPN ISOMER CONTAINING NICOTINAMIDE RIBOSIDE IN THE $\alpha$ LINKAGE[1]

*Sir:*

It has been reported previously that treatment of diphosphopyridine nucleotide (DPN) with the DPNase from *Neurospora crassa* results in the cleavage of the nicotinamide riboside bond.[2] Although the DPNase completely destroyed all activity of the DPN for yeast alcohol dehydrogenase, there was still some remaining reaction with cyanide.[3]

It was suggested that this cyanide reacting residue might be due to the presence of nicotinamide mononucleotide or riboside. We have recently reinvestigated this residue, and have found that DPN with high purity prepared from either liver or yeast still contains material which reacts with cyanide and is resistant to the action of the *Neurospora* enzyme. However, the residue is not nicotinamide mononucleotide or riboside since the compound does not promote the growth of *Hemophilus parainfluenzae*, which will grow on either the riboside or nucleotide as well as on DPN.[4] We have now been able to isolate a compound from a number of highly purified commercial DPN preparations, which give the same analysis for adenine, nicotinamide, ribose and phosphate as DPN, but has no alcohol dehydrogenase activity. The compound was obtained by first treating the DPN preparation with the *Neurospora* DPNase, and then separating the residual cyanide reacting material from the adenosine-diphosphate ribose by column chromatography. The compound was precipitated from acid acetone. We have tentatively named this compound "DPN isomer" because of its identical analysis with DPN. From 5 g. of Pabst DPN[5] 300 mg. of the "isomer" was isolated. We have estimated the concentration of the "isomer" to be from 10 to 15% in the purified DPN.

The isomer moves at the same $R_F$ as DPN on paper in a large number of solvents. Likewise it cannot be separated from DPN by column chromatography. The isomer can be distinguished from DPN not only by its inactivity in the alcohol dehydrogenase (yeast or liver), the muscle triosephosphate dehydrogenase and muscle lactic dehydrogenase systems, but also by its inability to serve as substrate for the *Neurospora* DPNase and as a growth factor for *Hemophilus parainfluenzae*. The compound reacts with hydrosulfite to give a product of reduction showing an absorption peak at 348 m$\mu$; furthermore, the cyanide addition product of the isomer has a maximum absorption at about 332 m$\mu$ as compared to the 325 maximum for DPN.

DPN can serve as the nicotinamide source for a nicotinamide requiring *Neurospora* mutant. However, the isomer does not promote the growth of the mutant (Table I). On heating the isomer for 30′ at 100° (*p*H 5), the nicotinamide ribosidic bond is cleaved; and this results in growth activity for the mutant. This and other evidence indicate that the pyridine component of the isomer is nicotinamide.

Treatment of the isomer with the snake venom pyrophosphatase results in the liberation of all the adenine as 5′-adenylic acid. The nicotinamide mononucleotide moiety of the isomer has a similar cyanide addition product as the parent compound

(1) Contribution No. 108 of the McCollum–Pratt Institute. Aided by Grant No. C-2374 C, from the National Cancer Institute of the National Institute of Health, The American Cancer Society as recommended by the Committee on Growth of the National Research Council and the American Trudeau Society Medical Section of the National Tuberculosis Association.

(2) N. O. Kaplan, S. P. Colowick and A. Nason, *J. Biol. Chem.*, **191**, 473 (1951).

(3) S. P. Colowick, N. O. Kaplan and M. M. Ciotti, *ibid.*, **191**, 447 (1951).

(4) W. Gingrich and F. Schlenk, *J. Bact.*, **47**, 535 (1944); N. R. Bachur and N. O. Kaplan, in preparation.

(5) We wish to thank the Pabst Laboratories for generously supplying some of the DPN used in these experiments.

TABLE I

EFFECT OF HEATING "DPN ISOMER" ON GROWTH OF NICOTINAMIDE REQUIRING NEUROSPORA MUTANT

0.04 of a micromole of each pyridine compound was added in a volume of 20 ml.; growth was for a period of three days.

| Additions | Dry weight of mat, milligrams |
|---|---|
| None | 0 |
| Nicotinamide | 107 |
| DPN | 73 |
| DPN Heated[a] | 103 |
| "DPN Isomer" | 0 |
| "DPN Isomer" heated[a] | 100 |

[a] Heated at 100° for 30 minutes. No reaction with cyanide occurred after heating, indicating complete cleavage of the N-glycosidic linkage.

(with an absorption maximum at 322 mμ.) Nicotinamide mononucleotide obtained from DPN has a maximum cyanide absorption at 325 mμ. The two mononucleotides also react at different rates with a number of different nucleotidases. Heating the two mononucleotides yields pentose phosphate products which are identical with ribose 5'-phosphate as determined by chemical and chromatographic procedures. The ribose phosphates also react at identical rates in the pentose isomerase and transketolase reactions of Horecker, *et al.*[6]

The fact that the isomer appeared to be different from "normal" DPN in the nicotinamide glycosidic linkage, led us to carry out optical rotation measurements of the mononucleotides. As can be seen from Table II, the two mononucleotides have completely different rotations. The isomer mononucleotide is positive rotating whereas the mononucleotide of DPN is negative rotating.[7] The difference is also observed in the dinucleotide form; the isomer has a rotation of +14° whereas DPN has a rotation of −35°. Such rotations might be expected since 5'-AMP has a negative rotation of about −40°. Heating the two mononucleotides yields products which give only a very slight rotation and which are quite similar. We therefore feel that the isomer contains nicotinamide riboside in the α position as contrasted to the beta nicotinamide ribosidic linkage of DPN.

TABLE II

OPTICAL ROTATIONS OF DPN AND DERIVATIVES

| | Compound | $[\alpha]^{23}D$ 1% in $H_2O$ |
|---|---|---|
| (1) | DPN | −34.8 |
| (2) | DPN isomer | +14.3 |
| (3) | Nicotinamide mononucleotide from (1) | −38.3 |
| (4) | Nicotinamide mononucleotide from (2) | +58.2 |
| (5) | Ribose 5'-phosphate from (3) | − 2.7 |
| (6) | Ribose 5'-phosphate from (4) | − 4.7 |
| (7) | 5'-Adenylic acid | −40.0 |

We cannot as yet state with certainty whether the isomer is formed during isolation or is a naturally occurring product. However, we have been able to detect the isomer in crude extracts from yeast and animal tissues, by treating the crude extracts with the *Neurospora* DPNase and then assaying for cyanide reacting material. It is of interest to note that the α isomer appears to have a somewhat more negative potential (closer to the hydrogen electrode at *p*H 7) than the beta isomer of DPN.[8] We are now investigating the possible biological significance of the compound. Details of the properties of the α isomer of DPN will be presented elsewhere.[9]

(6) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953).

(7) The values given in Table II have an accuracy of ±2 to 3°. The 5'-AMP present in both DPN and the isomer is of the beta glycosidic linkage.

(8) M. M. Weber and N. O. Kaplan, unpublished experiments. The potential was estimated from the end-point of an electron exchange reaction between the reduced isomer and oxidized DPN, catalyzed by a *Clostridial* enzyme.

(9) We wish to thank Dr. Joseph Riden for help in the optical rotation studies and Dr. B. L. Horecker for carrying out the transketolase test.

NATHAN O. KAPLAN
THE MCCOLLUM-PRATT INSTITUTE MARGARET M. CIOTTI
THE JOHNS HOPKINS UNIVERSITY FRANCIS E. STOLZENBACH
BALTIMORE 18, MARYLAND NICHOLAS R. BACHUR

RECEIVED DECEMBER 23, 1954

## STUDIES ON THE BIOSYNTHESIS OF ARTERENOL. ENZYMATIC DECARBOXYLATION OF DIASTEREOISOMERS OF HYDROXYPHENYLSERINES[1]

*Sir:*

Conflicting reports regarding the decarboxylation of 3,4-dihydroxyphenylserine (DOPS) by tissue extracts *in vitro* have appeared[2-7] although studies *in vivo*[7,8] point to the formation of arterenol. The availability of the diastereoisomers of DOPS,[9] *m*-hydroxyphenylserine (MOPS)[10] and *p*-hydroxyphenylserine (POPS),[10] and of a chromatographic method for assessing their homogeneity[11] have allowed reëvaluation of their activity.

*erythro*-DOPS is decarboxylated at an appreciable rate by hog kidney enzyme,[12] but to a lesser extent than DOPA, and *threo*-DOPS and *erythro*-MOPS are decarboxylated rather slowly (Table I). However, *threo*-DOPS and *erythro*-DOPS are decarboxylated at the same rate by whole liver homogenate.

Arterenol ($R_F$ 0.45) and α-aminomethyl-*m*-hydroxybenzyl alcohol ($R_F$ 0.62), respectively,

(1) This investigation was supported by grants from the Los Angeles County Heart Association, the U. S. Public Health Service (Grant 658), and the Life Insurance Medical Research Fund. We are indebted to Drs. J. M. Sprague, K. H. Beyer and W. A. Bolhofer, Sharp & Dohme, for the hydroxyphenylserine diastereoisomers, and to Dr. A. M. Lands, Sterling-Winthrop Research Institute, for the α-aminomethyl-*m*-hydroxybenzyl alcohol. The *erythro*-phenylserine was synthesized in this laboratory by Dr. R. I. Akawie, by the method of Y. Chang and W. H. Hartung, THIS JOURNAL, **75**, 89 (1953).

(2) H. Blaschko, P. Holton and G. H. Sloane Stanley, *Brit. J. Pharmacol.*, **3**, 315 (1948).

(3) K. H. Beyer, H. Blaschko, J. H. Burn and H. Langemann, *Nature*, **165**, 926 (1950).

(4) H. Blaschko, J. H. Burn and H. Langemann, *Brit. J. Pharmacol.*, **5**, 431 (1950).

(5) T. Sourkes, P. Heneage and Y. Trano, *Arch. Biochem. Biophys.*, **40**, 185 (1952).

(6) G. Fodor and J. Kiss, *Acta Univ. Szeged., Chem. et Phys.*, **3**, 26 (1950).

(7) K. H. Beyer, "Advances in Chem. Ser.," No. **2**, 37 (1950).

(8) C. G. Schmiterlöw, *Brit. J. Pharmacol.*, **6**, 127 (1951).

(9) W. A. Bolhofer, THIS JOURNAL, **76**, 1322 (1954).

(10) W. A. Bolhofer, *ibid.*, **75**, 4469 (1953).

(11) W. Drell, unpublished.

(12) O. Schales and S. S. Schales, *Arch. Biochem.*, **24**, 83 (1949).