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SHORT REPORTS

ABSENCE OF DIAMINOPIMELIC ACID FROM PLANT MATERIAL

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Key Word Index—*Pinus strobus*; Pinaceae; 2,6-diaminopimelic acid; absence from plants.

2,6-Diaminopimelic acid is a common constituent of bacteria and an intermediate in one of the pathways for lysine biosynthesis [1]. The presence of diaminopimelic acid in pine pollen has been reported on basis of paper chromatographic evidence [2]. Since this is the only report of the presence of diaminopimelic acid in higher plants and since the claim has acquired general acceptance [3–5] it was of interest to substantiate or disprove it.

In the original report "pine pollen (white and california)" were hydrolysed for 8 hr at 125° in 6 N HCl. After filtration, the solution was concentrated to dryness, treated with bromide, evaporated to dryness again, and an aliquot was subjected to PC on Whatman No. 1 paper in MeOH–H_2O–10 N HCl–pyridine (32:7:1:4). Diaminopimelic acid was identified by comparison with a reference spot and on basis of a characteristic olive green ninhydrin colour. It is however reported that the amino acid from pine pollen gave "a slightly different color reaction" from that of the reference sample but identical with that reported by other authors for diaminopimelic acid. The amount of pollen used and the amount of hydrolysate used for PC was not reported [2].

In order to assess the identification, 1 g pollen from white pine was hydrolysed by reflux for 24 hr with 6 N HCl. After evaporation to dryness and solution in 1 ml of water, 15 μl was subjected to PC in the system described above. A mixture of *meso*- and DL-diaminopimelic acid was used as reference. A spot was observed from the pine hydrolysate which showed some similarity with diaminopimelic acid. However, when 35.4 mg of the pollen were hydrolysed for 24 hr in 6 N HCl in a closed ampoule and the hydrolysis mixture subjected to automatic amino acid analysis on a Beckman/Spinco Model 120 Analyser, no diaminopimelic acid was found. The position of diaminopimelic acid on the chromatogram was found by chromatography of the diaminopimelic sample alone and in a mixture with the pine hydrolysate. This puts an upper limit of 0.05 μmol/g for the amount of diaminopimelic acid in pollen. Since 0.05 μmol is the amount of diaminopimelic acid necessary to produce a clear spot on PC, the amount of hydrolysate put on the paper in the original report should have corresponded to 1 g of pollen, if diaminopimelic acid should have been identified in this way. This is clearly impossible.

Finally 1 g of pollen was hydrolysed by reflux under N_2 with 6 N HCl for 8 hr. After concentration to dryness, an amino acid fraction was produced by binding to a strongly acid ion exchange resin in the hydrogen form with subsequent elution with aqueous pyridine. The whole fraction was subjected to preparative PC in the system described above. The band corresponding to the isomers of diaminopimelic acid was eluted from the paper, and after purification by binding to a strongly acid ion exchange resin in the hydrogen form and elution with aqueous pyridine, a fraction of 21.4 mg was obtained. PC with 200 μg of this material showed a spot with the same R_f as diaminopimelic acid but with somewhat different colour reaction with ninhydrin. 100 μg of the sample was subjected to quantitative amino acid analysis. No diaminopimelic acid could be observed, whereas a number of other amino acids including aspartic acid, serine, glutamic acid, and glycine were present. The upper limit for diaminopimelic acid present in 1 g of pollen can be estimated to 0.10 μmol from this experiment.

On this basis we conclude that diaminopimelic acid is not present in pine pollen and that in the earlier identification other amino acids have been responsible for the spot attributed to diaminopimelic acid.

The biosynthesis of lysine in higher plants has never been established with certainty, but recent investigations [6,7] indicate that the diaminopimelate pathway is operating. It is, however, remarkable that diaminopimelic acid never has been identified in plant material.

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ϵ-*N*-TRIMETHYLLYSINE DESAMINASE DE *NEUROSPORA CRASSA*

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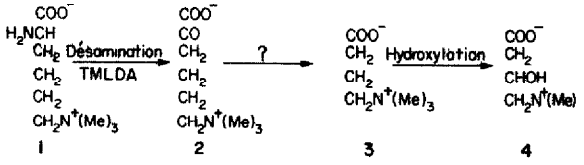
Key Word Index—*Neurospora crassa*; fungus; ϵ-*N*-trimethyllysine deaminase; ϵ-*N*-trimethyllysine; 2-keto-6-*N*-trimethylhexanoic acid.

Abstract—ϵ-*N*-Trimethyl-L-lysine is transformed into 2-keto-6-*N*-trimethylhexanoic acid by a soluble deaminase present in the mycelium of *Neurospora crassa*. The activity of the enzyme increases with the age of the mycelium with an optimum after 10 days of culture. ϵ-*N*-Trimethyllysine deaminase has been purified 200-fold by differential centrifugation and successive column chromatography on DEAE Sephadex and Ultrogel AcA-34. Some of its properties have been studied. This is the first enzyme reported to metabolise ϵ-*N*-trimethyllysine.

INTRODUCTION

Nous avons récemment isolé à partir de *Neurospora crassa*, l'acide 2-céto-6-*N*-triméthylhexanoïque (**2**) et à l'aide de la ϵ-*N*-triméthyl ($^{14}CH_3$) lysine montré par des expériences *in vivo* et *in vitro* que ce céto-acide provenait bien de la ϵ-*N*-triméthyllysine (**1**) (TML). Ces expériences ont permis en même temps la mise en évidence d'un système enzymatique capable de désaminer la TML [1,2]. La détection de l'acide 2-céto-6-*N*-triméthylhexanoïque dans le mycélium de *Neurospora crassa*, laisse penser qu'il est un intermédiaire dans la biosynthèse de la carnitine (**4**), qui provient aussi de la TML via butyrobetaine (**3**) [3,4]. Nous avons entrepris la purification de la ϵ-*N*-triméthyllysine désaminase (TMLDA) afin d'essayer de préciser si elle joue un rôle dans la biosynthèse de la carnitine ou un rôle régulateur de la concentration intracellulaire de la TML.



Nous rapportons ici nos résultats préliminaires sur la purification et quelques unes des propriétés de cette désaminase, isolée à partir de *Neurospora crassa*. A notre connaissance c'est la première enzyme intervenant dans le métabolisme de la TML mise en évidence.

RESULTATS ET DISCUSSION

TMLDA a été isolée à partir du mycélium de *Neurospora crassa* cultivé pendant dix jours. Des essais préalables ont montré que l'activité enzymatique augmentait avec l'âge de la culture (avec un rendement optimal au dixième jour).

L'enzyme a été purifiée par centrifugation différentielle de l'extrait obtenu par broyage avec du sable dans un mortier en présence de K_2HPo_4 0,1 M; le surnageant est chromatographié sur une colonne de DEAE Sephadex-50 (équilibrée avec le même tampon) qui retient l'enzyme; l'activité enzymatique est éluée avec du tampon phosphate contenant 0,4 M de KCl. Les fractions contenant l'enzyme sont réunies et précipitées par le $(NH_4)_2SO_4$ à 90%; le précipité, récupéré par centrifugation, est redissous et chromatographié sur colonne d'Ultrogel AcA-34 et élué avec due phosphate 0,1 M. Les fractions contenant la désaminase sont réunies. Les résultats de ce procédé de purification sont indiqués dans le Tableau 1. La préparation enzymatique obtenue après ces manipulations possède une activité spécifique environ 200 fois supérieure à celle de l'extrait de départ.

Une relation linéaire entre l'activité enzymatique et le temps d'incubation est obtenue pour des temps allant de 0 à 75 min. Un temps d'incubation de 30 min a été choisi pour nos expériences. L'activité optimale est obtenue vers pH 9,5; cependant toutes nos expériences ont été réalisées à pH 8,9.

Une série d'incubations avec des concentrations initiales différentes de TM ($^{14}CH_3$)L a été effectuée afin de déterminer le $K_{m_{app}}$. Il est de 0,33 mM, calculé à partir de la représentation de Lineweaver–Burk. L'introduction de KCl dans le milieu d'incubation stimule l'activité enzymatique avec un maximum à 0,8 M (Tableau 2).

Des expériences en vue de préciser le mécanisme d'action de la TMLDA ont été réalisées; ainsi nous avons testé le FAD, FMN, NAD et le Pyridoxal phosphate