synthesis. It should also be noted (Figure 2) that the carotenoids produced in the dark grown, cycloheximide, and AM inhibited cultures are the 'early' polyenes while the induced controls had proportionately more of the 'later' carotenoids; neurosporene, $\gamma$-carotene and some lycopene.

These results can be interpreted by assuming that *Neurospora* constitutively synthesizes a low level of dark synthesized carotenogenic proteins which can be increased 10 fold by photoinduction. The results indicate that if there is a photosensitive control molecule, it must persist in its effective state for at least 1 h.

Cycloheximide is a potent translation inhibitor and completely blocks the synthesis of newly induced carotenogenic proteins. AM on the other hand inhibits only 92% of the transcriptional activity of *Neurospora crassa*[11] and presumably the residual 8% is responsible for the incomplete inhibition of carotenogenesis.

These results do indicate however, that carotenogenesis proceeds by the de novo transcription of new m RNA rather than the translation of pre-existing m RNA as is the case with some other regulatory systems[16].

Wheter the photosensititive control moiety is a photosensitive repressor using light as a deactivating agent (similar to the galactoside allosteric inhibition of the *lac* repressor according to JACOB and MONOD[17]), or whether light acts as activating agent for a photosensitive control moiety responsible for initiation of transcription for specific gene sequences or batteries of genes (according to BRITTEN and DAVIDVON[18]), cannot be resolved at this time[19].

*Résumé. Neurospora crassa* produit constitutivement un faible taux de caroténoïdes. Celui-ci peut être élevé dix fois par photoinduction. Nous avons trouvé que l'actinomycine D inhibe cette synthèse photoinduite des caroténoïdes probablement au niveau de la transcription des protéinse caroténogènes.

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## Determination of C-Terminal Group of Aspergillopeptidase Af

The physico-chemical properties, composition of amino acids in isolated extracellular endopeptidase Af from liquid culture *Aspergillus fumigatus* was described. It was also determined that the N-terminal groups of these enzyme are glycine and glutamic acid[1]. On the basis of this work, it may be supposed that the isolated enzyme is a polypeptide compound of 2 chains. The purpose of this report was to analyse the C-terminal amino acids of the enzyme in order to determine the 2 chain structure of this protein.

*Material and methods.* The material used was lyophillizing enzyme preparation isolated from the Čapka-Dox liquid culture from *Aspergillus fumigatus*. The method of isolation and purification was described earlier[1]. C-terminal amino acid analysis was carried out by the hydrazine method according to BRADBURY[2-4]. 50 mg of enzyme and 130 mg $N_2H_4H_2SO_4$ was dried in room temperature for about 3 h in vacuum. 1 ml of 100% of previously distilled hydrazine was added, the whole sealed in test tube and heated at 60 °C for 16 h. The excess of hydrazine was evaporated over concentrated $H_2SO_4$ in vacuum.

The remander was dissolved in 0.1 *N* HCl. The solution was shaken with 2.0 ml benzaldehyde for 2 h. The water solution was separated by centrifugation. In 3 ml of the solution, 0.1 ml 3.1 *M* KCl was added, and pH was adjusted to 9.0 by the addition of 40 µmol 0.20 *N* NaOH. The solution was saturated with 1, 2, 4-fluorodinitrobenzene at 40 °C by energetic mixing (by excess reagent) at constant pH 9 for 80 min. The solution was extracted using ether free from peroxide in order to remove FDNB. The remainder was acidized with 5 N HCl. DNP amino acids were extracted 5 times with ether. As a result of extraction, 2 fractions were obtained, ether and water. Both fractions were analyzed to detect the presence of DNP amino acids by placing 200 µl concentrated extract on a sheet of Whatman no 1. Analysis of water and ether

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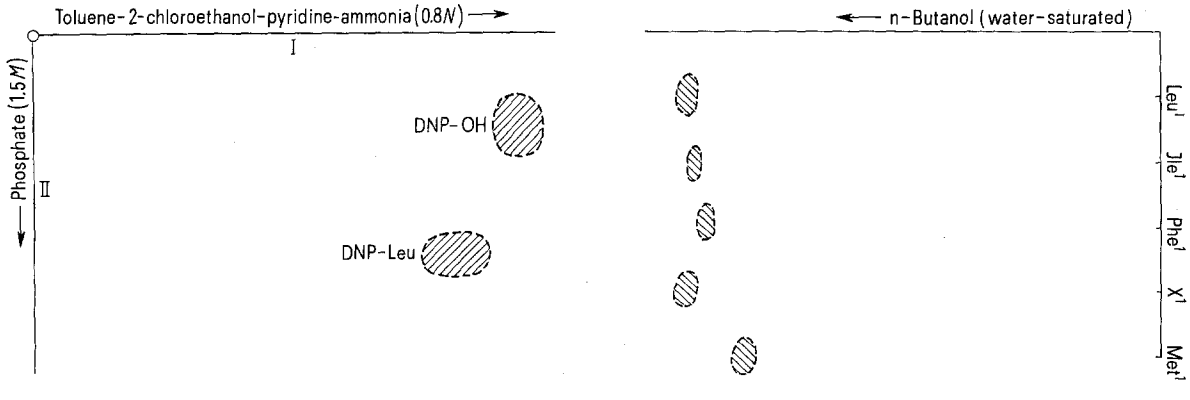

extract was done by the Bisert two-dimensional paper chromatography method[5] using in the first direction (ascending dimension): toluene-pyridine-ethylene chlorhydrin (2chloroethanol)-ammonia 0.8 *N* at the rate of 5:3:1.5:3. In the second direction for descending chromatography with a solvent system consisting of a 1.5 *M* phosphate buffer of pH 6: 1 *M* $NaH_2PO_4$, 0.5 *M* $Na_2HPO_4$; i.e. 138 g of $NaH_2PO_4$ $H_2O$ and 71 g $Na_2HPO_4$ per l.

In order to obtain univocal results, the DNP amino acid derivatives were eluated with independent methanol and 1% $Na_2CO_3$, and also subjected to rechromatographic process using water saturated butanol (according to MELLON, KORN, HOOVER[6]). Rechromatography was carried out parallel to amino acid standards for identification purposes.

*Results.* By the method of two-dimensional chromatography (ascendance and descendance) using hydrazinolyse technique, compounds were obtained, one of which Rf corresponded to dinitrophenyl, while the other, although twice subjected to chromatographic analysis, compared with standard amino acids turned out to be DNP leucin. The Table shows Rf DNP amino acids compared with the unidentified C-terminal amino acids. On Figure 1 are shown 2 DNP amino acid spots: dinitrophenyl and DNP leucine. Figure 2 shows the results of the division of rechromatographic standards of DNP amino acids and the analyzed DNP amino acids.

Comparison of Rf value examined and standard DNP amino acids

| Amino acids | Rf |
|---|---|
| Di-DNP Tyr | 0.78 |
| DNP Leu | 0.74 |
| DNP Ile | 0.73 |
| Di-DNP-Lys | 0.72 |
| DNP-Phe | 0.71 |
| DNP-Met | 0.65 |
| DNP-Val | 0.68 |
| DNP-X | 0.74 |
| X-examined amino acid | |

*Discussion.* On the basis of analysis carried out on C-terminal amino acids of aspergillopeptidase Af, a DNP-derived amino acid was obtained. This was found to be leucine. This result suggests that the isolated enzyme possesses two identical C-terminal amino acids, if we assume that the molecule has a two chain structure. It may be that the enzyme has 1 polypeptide chain of branched structure. This is indicated by the presence of two N-terminal amino acids.

*Résumé.* Nos observations concernent la détermination de groupes C-terminaux d'aspergillopeptidase Af extraite de la culture d'*Aspergillus fumigatus*. Nous avons utilisé la technique de l'hydrazinolyse et avec la chromatographie alternante et démontré que la leucine constitue un amino-acide C-terminal.

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## Nachweis von radioaktiven Aminosäuren im Kollagen nach Gabe von Prolin-$C^{14}$

Eigene Untersuchungen haben ergeben, dass im Körper der Ratte aus dem Prolin nicht nur Hydroxyprolin und Glutaminsäure, sondern auch Glykokoll, Alanin, Serin und Threonin entstehen[1]. Bekanntermaßen handelt es sich um Aminosäuren, die im Kollagen festzustellen sind. Es ergibt sich die Frage, inwiefern sie nach Prolin-$C^{14}$-Gabe im erwähnten Skleroprotein erscheinen.

*Material und Methode.* Männliche, ca. 120 g schwere Sprague-Dawley-Ratten bekamen je 500 µCi L-Prolin-U-$C^{14}$ (spez. Aktivität 209 mCi/mMol) i.p. und wurden 3, 24, 48 und 72 h später getötet. Die frei von Haaren und Fett präparierte Haut ebenso wie die Schwanzsehne der Tiere wurden getrennt, gewaschen, in 0,5 *M* Natriumazetatpuffer homogenisiert, in der Kälte geschüttelt und bei 2000 upm zentrifugiert. Nach Dekantierung, nochmaliger Gabe von Natriumazetat, 36 h bei 5° Schütteln und anschliessender Zentrifugierung wurde der Rückstand mit Zitratpuffer (pH 4,3) 72 h bei 5°- geschüttelt. Den filtrierten Extrakt zentrifugierten wir bei 50000 × *g*. Anschliessend wurde der Überstand in 0,02 *M* $Na_2HPO_4$ Puffer bei 5 °C rund 3 Tage lang dialysiert[2]. Der während der Dialyse entstandene Niederschlag war lichtmikroskopisch als nadeliges Element zu identifizieren, das elektronenmikroskopisch die typische Struktur der ausgefällten mit entsprechenden Querstreifen versehenen kollagenen Fasern aufwies. Die gewaschenen und getrockneten Kollagenfibrillen zeigten eine für dieses Kollagen[3,4] charakteristische Aminosäurenzusammensetzung (% µMol):

| | Haut | Sehne |
|---|---|---|
| Alanin | 10,50 ± 0,23 | 10,77 ± 0,37 |
| Arginin | 5,66 ± 1,36 | 5,14 ± 0,39 |
| Asparaginsäure | 4,98 ± 0,51 | 5,04 ± 0,38 |
| Glutaminsäure | 6,78 ± 0,39 | 7,03 ± 0,19 |
| Glyzin | 30,77 ± 1,46 | 31,60 ± 0,74 |
| Histidin | 0,65 ± 0,13 | 0,63 ± 0,23 |
| Hydroxylysin | 0,73 ± 0,19 | 0,72 ± 0,05 |
| Hydroxyprolin | 11,95 ± 1,40 | 11,41 ± 1,03 |
| Leuzin | 2,36 ± 0,27 | 2,40 ± 0,22 |
| Iso-Leuzin | 1,22 ± 0,13 | 1,28 ± 0,23 |
| Lysin | 3,28 ± 0,65 | 2,87 ± 0,05 |
| Methionin | 0,30 ± 0,15 | 0,29 ± 0,07 |
| Phenylalanin | 1,22 ± 0,14 | 1,20 ± 0,03 |
| Prolin | 11,30 ± 0,42 | 11,87 ± 0,22 |
| Serin | 3,79 ± 0,31 | 3,77 ± 0,23 |
| Threonin | 1,93 ± 0,29 | 1,95 ± 0,15 |
| Tyrosin | 0,29 ± 0,11 | |
| Valin | 2,49 ± 0,42 | 2,26 ± 0,29 |