NOTE

A Substance Accelerating Amidase Activity of Trypsin in Autoxidized Methyl Linolenate

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A substance which accelerates the amidase activity of trypsin by more than three times that of intact trypsin was separated from autoxidized methyl linolenate by gel chromatography on Sephadex LH-20, silicic acid chromatography on Silica gel 60, and by high-performance liquid chromatography on μ -Porasil. This substance isolated from the highly active fraction was identified as methyl 12-oxo-9-hydroxy-10-dodecenoate by GLC and GC-MS analyses.

1 Introduction

Although the inactivating effects of oxidized lipids on enzymes are well known^{1),2)}, little is known of their accelerating effect. Matsushita *et al.*^{3),4)} studied the interaction between the oxidized products of linoleic acid and pepsin and found that the proteinase activity of pepsin was accelerated by the reaction with linoleic acid hydroperoxides and with their degradation products. However, the substance responsible for the acceleration was not identified. In a preceding paper⁵⁾, we reported that a group of degradation products of autoxidized methyl linolenate (oxd MLn) selectively accelerated the amidase activity of trypsin, and that this acceleration was primarily based on the reaction between the ϵ -amino group of trypsin and the carbonyl group of the degradation products of oxd MLn. In the present work, we separated and identified the main accelerating substance in oxd MLn.

2 Results and Discussion

2.1 Separation of accelerating substances from oxd MLn

MLn (Tokyo Kasei Kogyo Co.) was autoxidized at 40°C for 4 d in the dark, and the degradation products separated by chromatography on a Sephadex LH-20 column, using acetone as the eluent⁵⁾, followed by a Silica gel 60 prepacked column (24×1 cm E. Merck Co.) with CHCl₃ : MeOH (97/3, vol/vol) as the eluent⁶⁾. The degradation products had a po-

tent accelerating effect on the amidase activity of trypsin and were further separated by high performance liquid chromatography (HPLC) on a μ -Porasil column (30×0.78 cm, Waters Assoc. Inc.) with *n*-hexane : 2-propanol (95/5, vol/vol) as the eluent (4.0 mL/min)⁶⁾.

Fig.-1 shows the result of HPLC applied

Fig.-1 High-performance liquid chromatography of the degradation products prepared by silicic acid chromatography.

Table-1 Accelerating effect of HPLC-fraction* on amidase activity of trypsin.

Fraction	A	B	C	D	E	F	G	H	I	J
Acceleration (%)**	104	93	114	186	143	141	381	148	109	103

* Refer to Fig.-1. ** The enzyme activity of trypsin measured without adding oxd MLn was taken as 100%. Each fraction was allowed to react with 7 μ g of bovine pancreatic β -trypsin in 200 μ L of the mixture of EtOH and 0.1 M Tris-HCl buffer, pH 8.0 (10/90, vol/vol) containing 0.05 M CaCl₂ at 37°C for 1 h. Amidase activity of the reaction mixture was measured using α -N-benzoyl-DL-arginine-p-nitroanilide as the substrate.

degradation products prepared by silicic acid chromatography. All peaks were recovered, as illustrated in **Fig.-1**. The accelerating effect of each fraction was assayed as described⁵⁾ (**Table-1**). Fraction G had the most potent accelerating effect on the amidase activity of trypsin. Therefore, fraction G was re-chromatographed at the same HPLC condition until a single peak was obtained. The recovery of purified fraction G was about 0.4% from the starting oxd MLn.

2.2 Identification of the main accelerating substance

After reduction with NaBH_4 and the subsequent trimethylsilylation, the substance stimulating the amidase activity in fraction G was analyzed by GLC and GC-MS, as described⁶⁾. Only one prominent peak (ECL 17.5, determined using authentic fatty acid methyl esters⁷⁾) is observed in the chromatogram (**Fig.-2**), thereby indicating the homogeneity of fraction G. The principal ions and their relative intensities of this peak obtained by EI-mass spectrometry at 20 eV were as follows: m/z 373 (M-15, loss of CH_3), 7.0%; 357 (M-31, loss of CH_3O), 7.0; 298 (M-90, loss of HO-TMS), 9.0; 285 (M-103, loss of CH_2OTMS), 27; 259 (α -cleavage at 9 C-OTMS), 6.5; 231 (α' -cleavage at 9 C-OTMS), 100; 191, 9.5; 169 (259-90), 5.6; 155, 13; 147, 9.2; 129, 7.0; 75, 18; 73, 21; 69, 34. The fragmentation pattern was in close agreement with that of the TMS ether of methyl 9,12-dihydroxy-10-dodecenoate, already reported by our group⁸⁾. We found that

Fig.-2 Gas chromatography of NaBH_4 -TMS ethers derived from fraction G.

Fig.-3 EI-mass spectrum of NaBD_4 -TMS ether of a principal component in fraction G.

the methyl 9-hydroxy-12-oxo-10-dodecenoate, the mother compound of this TMS-derivative, was the amino acid-reactive substance formed during autoxidation of methyl linoleate. Further evidence for the presence of the hydroxyaldehyde was obtained by GC-MS analysis of the NaBD_4 -TMS ether (**Fig.-3**). Deuterium containing fragment ions, such as, m/z 374 (M-15), 358 (M-31), 299 (M-90), 232 (α' -cleavage of 9 C-OTMS), and non-deuterium containing ions like m/z 259 (α -cleavage at 9 C-OTMS) and 285 (M-104) indicate the position of hydroxyl and formyl group in the molecule. Thus, the accelerating substance in fraction G proved to be methyl 9-hydroxy-12-oxo-10-dodecenoate.

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自動酸化リノレン酸メチル中のトリ プシンのアミダーゼ活性促進物質

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自動酸化リノレン酸メチルからトリプシンのアミダーゼ活性を3倍以上促進する物質を, Sephadex LH-20 ゲル濾過法, Silica gel 60 ケイ酸クロマト法及び μ -Porasil HPLC で分離・精製した。同物質を GLC 及び GC-MS で分析し, 9-ヒドロキシ-12-オキソ-10-ドデセン酸メチルと同定した。

国際油脂情報

☆ 英国の多不飽和脂肪酸学会, 135 人参加

1986 年 12 月 11 日, ロンドンで開催された英国化学工業協会主催の表記学会は参加者 135 人に達した。コンサルタントの R.D. Rice 氏は EPA その他の $n-3$ 脂肪酸につき講演した。EPA, DHA の化学, 伝染病学及び薬学につき論じ, 1850 年から 1980 年までに $n-6/n-3$ 比が5倍も増加し, 不均衡が生じているとした。B.F. Blank は月見草, ルリデシャ及び黒スグリの種子中の γ -リノレン酸源としての考察, 及び種による種子の収量について述べた。Sturge Biochemical 社の P.E. Milson 氏は発酵法による γ -リノレン酸の生産につき報告し, 低窒素, 高炭水化物含量下の培地で γ -リノレン酸に富む油脂が蓄積されるという。また Efamol Ltd. M.A. Crawford, Nuffield Lab. of Comparative Medicine の D.F. Horrobin 氏は細胞機能の制御役と構成脂質としての $n-3$ 脂肪酸の役割につき講演し, 人間の食事とその脂肪酸組成の変化の歴史について述べた。

(INFO 1987 年 1 月号, 吉富和彦)

☆ ガット交渉, ジュネーブで開催

1987 年ガット交渉は 2 月 16 日, ジュネーブで開催された。農業関連がおもな議題で, なかでも油糧種子が重要な要素を占める。米国の観測筋は EEC の油脂に対する関税案に注目している。

(INFO 1987 年 2 月号, 吉富和彦)

☆ ADM と Gold Kist, 落花生企業で合併

ADM 社と Gold Kist 社はアトランタをベースに落花生の売買や脱殻業を行っている Golden Peanut 社の操業に対し, 協同事業に踏み切ると発表した。脱殻能力は年間 50 万トンと推定され, 米国全体の 40% を占める。

(INFO 1987 年 2 月号, 吉富和彦)

☆ イタリアの製油工場, 爆発で 3 人死亡

イタリア中部の Livorno にある Italso 油脂抽出工場で爆発が起こり, 3 人が死亡, 多数のけが人がでた。当局はイタリアにおける新鋭工場のひとつで起きた爆発と火災について原因を調査中である。

(INFO 1987 年 2 月号, 吉富和彦)