行政院國家科學委員會專題研究計畫成果報告

石蓴過氧化物水解酶之性質與純化

(Purification and Characterization of Ulva Hydroperoxide Lyase)

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主持人：郭建民　執行機構：嘉南藥理學院食品衛生系

一、中文摘要

本研究乃探討石蓴過氧化物水解酶(HPLS)之特性。石蓴粗抽出液經 MacroPrep-Q 離子交換及 Sephacryl S-300 膠過濾後，過氧化物水解酶純化了 31.7 倍。石蓴 HPLS 的最適 pH 及溫度分別為 pH 6.6 及 34℃，於 pH 6～8、溫度 37℃以下，HPLS 活性安定。對於不同脂肪酸過氧化物之反應性以 18：2-13OOH 及 18:3-13OOH 最高，對 18：2-9OOH 及 18:3-9OOH 不具反應性。探討八種不同過氧化物水解酶抑制劑之影響，以 quercetin 之抑制效果最高，其次為 $HgCl_2$, BHA, PG, esculetin, NDGA, BHT 及 esculin。

關鍵詞：*過氧化物水解酶、海藻、石蓴、純化。*

Abstract

Hydroperoxide lyase (HPLS) was isolated and purified 32-fold from sea algae (*Ulva lactuca)* using MacroPrep-Q ion exchange and gel filtration on Sephacryl S-300. The optimal pH of the algal HPLS was 6.6, and optimal temperature was 34 °C. At pH ranged 6 to 8 and temperature below 37 °C, the algal HPLS was stable. The algal HPLS showed the highest reactivity toward 18:2-13OOH and 18:3-13OOH, while no activity was observed on 18:2-9OOH and 18:3-9OOH. Eight synthetic HPLS inhibitors including BHT, BHA, quercetin, esculetin, esculin, NDGA, PG, and $HgCl_2$ were used in this study. Among them, quercetin showed the highest inhibition, followed by $HgCl_2$, BHA, PG, esculetin, NDGA, BHT and esculin.

***Keywords**: Hydroperoxide lyase, sea algae, Ulva lactuca, purification.*

二、緣由與目的

市售水產香料之香氣物質較不足，如何強化此等水產香料使其更接近海鮮香氣，仍值得研究。為強化市售水產香料之香氣，探討具水產或海鮮香氣之化合物或形成機制，可能是未來此一領域之研究發展的重點。(Pan and Kuo, 1994)

生鮮魚味之香氣成分可由不飽和脂肪酸經脂氧合酶與過氧化物水解酶催化作用產生。植物香氣之形成亦與脂氧合酶與過氧化物水解酶有關，植物成熟時散發之香氣成分，主要與脂氧合酶與過氧化物水解酶代謝脂質有關。魚漿中加入植物之脂氧合酶粗抽出液會產生強烈之鮮魚味 。以脂氧合酶粗抽出液處理 22:6 後，於微波加熱過程形成柴魚味。烏魚鰓之脂氧合酶粗抽出液亦被應用於修飾魷魚油或烏魚子味道或形成水產香氣。然而有關過氧化物水解酶於水產品中之探討則較少。

於前幾年之研究中 (NSC-84-2214-E-041-002、NSC 85-2214-E-041-001、 NSC 86-2214-E-041-004、NSC 87-2214-E-041-001) 發現兩種綠藻，石髮 (*Enteromorpha intestinalis*)與石蓴(*Ulva lactuca*)之脂氧合酶活性極高；其特性及與水產風味之關係已經探討。(Kuo and Pan, 1991, 1992, Pan and Kuo, 1994, Kuo, et al., 1994, 1996, 1997)。該酵素目前已應用於修飾魚油氣味之研究上(胡，1998)，然而石蓴或石髮中過氧化物水解酶之特性則未作探討。

本年度計畫中將純化及探討石蓴過氧化物水解酶之特性，以作為日後應用此二酵素於形成水產香氣系統之基礎。藉海藻脂氧合酶與過氧化物水解酶形成水產香氣，將可大為提高海藻之利用價值。由水產香氣物質形成之途徑

及最適條件，或可作為未來發展水產香料之參考。

三、結果與討論

石蓴粗抽出液經 MacroPrep-Q 離子交換及 Sephacryl S-300 膠過濾後，過氧化物水解酶活性(HPLS)純化了 31.7 倍，回收率為 19.7%（表 1）。於純化過程之 MacroPrep-Q 離子交換層析圖如圖 1 所示，HPLS 活性位於洗析體積 200-250 mL 之間。Sephacryl S-300 膠過濾層析圖如圖 2 所示，HPLS 活性位於洗析體積 54-64 mL 之間。石蓴 HPLS 的最適 pH 及溫度分別為 pH 6.6 及 34℃，於 pH6～8、溫度 37℃以下，HPLS 活性安定（圖 3 及 4）。該 HPLS 對於不同脂肪酸過氧化物之反應性以 18：2-13OOH 及 18:3-13OOH 最高，對 18：2-9OOH 及 18:3-9OOH 不具反應性（表 2）。故石蓴之 HPLS 屬於 13-HpOOH lyase，與蕃茄、茶葉、青椒等之 HPLS 類似 (Fauconnier, et al., 1997)。使用 8 種不同抑制劑探討活性之消長情形如表 3 所示。其中以 quercetin 及 $HgCl_2$ 之抑制效果最高，而 NDGA 及 esculetin 原為脂氧合酶強力抑制劑，但對石蓴 HPLS 之抑制效果，則並不高。

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六、自評

本研究係有關海藻過氧化物水解酶之首篇報告，並已順利完成計畫之目標。由於石蓴之風味佳，應用潛力大，而影響其風味之主要酵素為 LOX 及 HPLS。若對 HPLS 之特性有充分瞭解將有助於工業上應用此類風味酵素之參考。而後續將混合幾種風味相關酵素，建立生化反應器以形成水產香氣。

五、參考文獻

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Table.1 Purification of hydroperoxide lyase from sea algae (*Ulva lactuca*).

stage	total activity (μmole hexanal /min)	total protein (mg)	specific activity (μmole hexanal /mg-min)	recovery (%)	purification (fold)
crude extract	112.8	745.1	0.15	100	1.0
MacroPrep-Q	32.3	11.8	2.73	28.6	18.2
Sephacryl S-300	22.2	4.7	4.76	19.7	31.7

a: The HPLS activity was assayed by headspace analysis and the decrease in absorbance at 234 nm.

Table 2. Substrate specificity of the partial purified algal hydroperoxide lyase.

Fatty acid hydroperoxide	Relative activity (%) [a]
18:2-13-OOH [b]	100
18:2- 9-OOH [c]	2
18:3-13-OOH [b]	92.5
18:3- 9-OOH [c]	4

a: The HPLS activity was assayed by the decrease in absorbance at 234 nm.

b: The hydroperoxides were prepared from 18:2 and 18:3 treated with soybean LOX.

c: The hydroperoxides were prepared from 18:2 and 18:3 treated with tomato LOX.

Table.3. Effects of various inhibitors on the activity of partially purified algal hydroperoxide lyase.

inhibitor	IC_{50} (μM) [a]
BHT	1500
BHA	200
quercetin	10
esculetin	500
esculin	1500
NDGA [b]	800
PG (propyl gallate)	500
$HgCl_2$	50

a: The concentration of inhibitor causing 50 % reduction of enzyme activity.

b: nordihydroguaiaretic acid

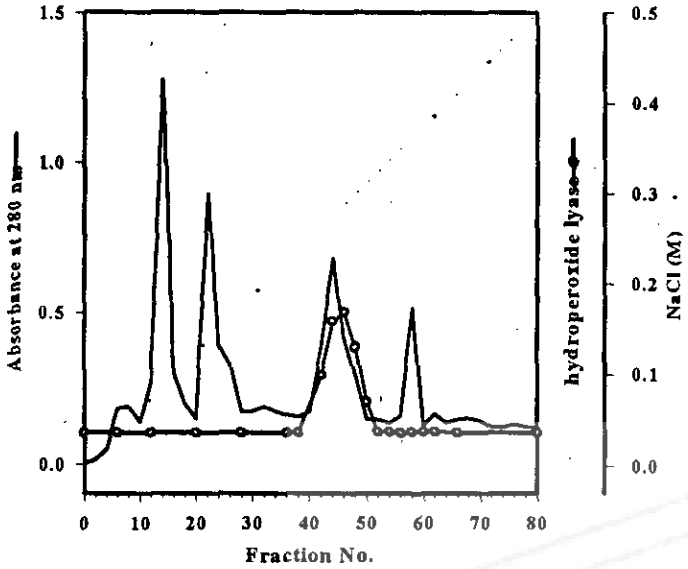

Fig.1 Elution profile of Ulva lactuca HPLS on Macro Prep Q column (4.4 X 17 cm). Column was equilibrated with 50 mM tris buffer (pH 6.4) and eluted with a linear gradient of 0 to 0.5 M NaCl at a flow rate of 0.8 mL/min. Fractions in 5 mL were collected and assayed for protein as absorbance at 280 nm and for HPLS activity.

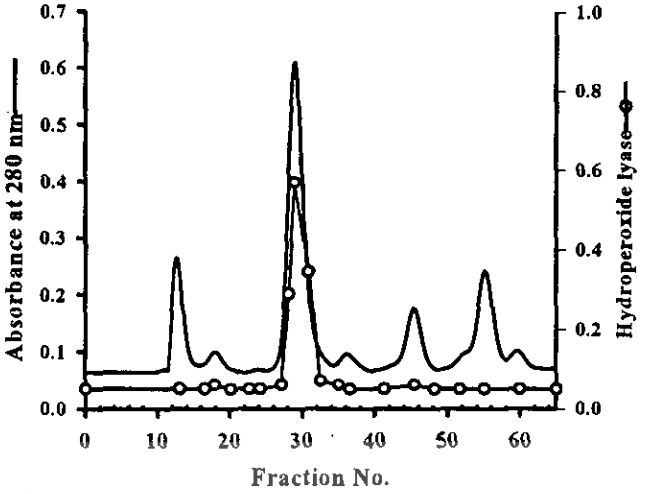

Fig.2 Elution profile of Ulva lactuca HPLS on Sephacryl S-300 column (1.6x90 cm) Column was equilibrated with 50 mM tris buffer (pH 6.4) and eluted with the same buffer at a flow rate of 0.3 mL/min. Fractions in 2 mL were collected and assayed for protein as absorbance at 280 nm and for HPLS activity.

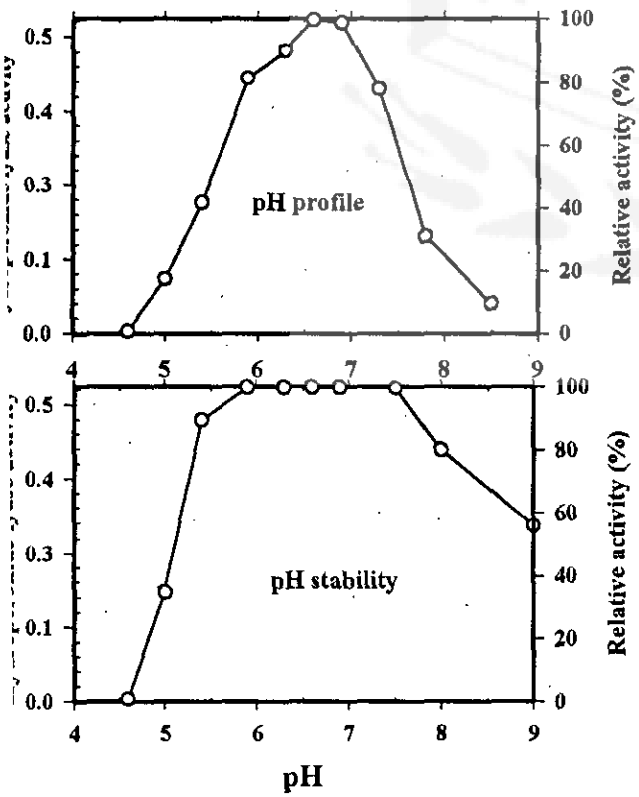

Figure 3. pH profile and pH stability of algal HPLS activity. The pH stability was performed by preincubatimg algal HPLS at different pH for 10 min and then assayed for HPLS activity.

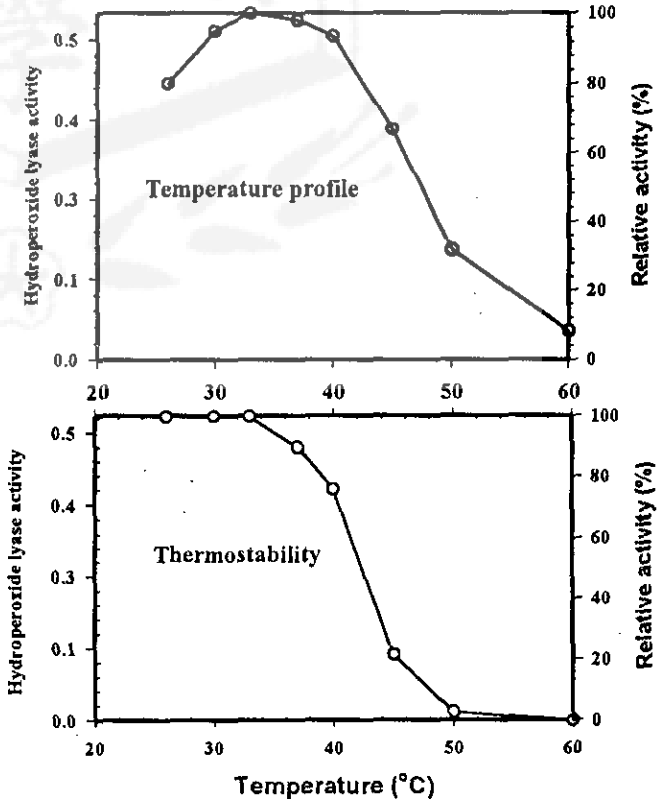

Figure 4. Temperature profile and thermostability of algal HPLS. The thermostability was performed by preincubatimg algal HPLS at different temperature for 10 min and then assayed for HPLS activity.