鯉魚之生態、生理與其高濃度之鋅

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1. 鯉魚之生態

魚類是最多樣性的脊椎動物。相對於其他脊椎動物，魚類之演化地位及其能夠適應廣泛不同環境，使得魚類在環境生物學，內分泌學，發生學及許多領域上，都是極佳的實驗模式動物。由於魚類生存之環境常有溫度、氧氣、壓力、pH、鹽度等變化，比較生理學者及生化學者極有興趣了解魚類如何調整及適應外界環境之壓迫。鯉魚原產於中國，後傳入近東及日本，再傳至歐洲、北美、中南美、澳洲、南非及印度，幾乎世界各地皆有鯉魚棲息。鯉魚之所以能在如此不同地區繁殖，與其之生態特性極有關係。鯉魚雖然性喜溫暖 (20 ℃以上)，但可容忍極低，極久之低溫 (接近 0 ℃)，亦可容忍溫度之驟變。在水中完全無氧的狀態下，多數鯉魚 (>80%) 至少可存活 5 日;在低氧狀態 (1-2 ppm 溶氧)，則能生存數週至數月。鯉魚主要以底棲生物，及浮游動物為餌；池底的螺、貝、昆虫與紅筋虫等均喜攝食；但亦攝食植物種子，水生植物，底屑等。鯉魚之近親，鯽魚與金魚，同樣地亦具有上述 一 耐低溫，低氧，雜食等生態特性。另外，鯉魚、鯽魚 (金魚) 可能因為上述特性，極為長命。養殖之鯉魚，鯽魚 (金魚) 超過 50 歲者，極為平常。

2. 鯉魚高濃度鋅之發現

鋅是生物必須之微量元素。在許多哺乳動物，如人、猴、鼠、牛及羊，其多數組織之鋅濃度在 10-100 μg/g wet weight 之間，而不同種屬之間差異甚小。鳥類之鋅濃度與哺乳類相近。魚類肌肉部份之鋅濃度平均只有 6.5 μg/g wet weight，其範圍在 3-24 μg/g wet weight 之間。為了解台灣養殖魚貝類是否受到重金屬污染，我們乃自台灣不同養殖地區，採購鯉魚、草魚、鰱魚、吳郭魚及其他魚類，分析其重金屬含量，結果發現台灣之養殖魚貝類一般說來，並未受到重金屬污染。在所有魚類之各種組織中，鋅之濃度在 10-50 μg/g fresh tissue 之間。然而鯉魚內臟鋅之濃度，卻與眾不同，高達 174-585 μg/g wet weight。進一步詳細研究台灣產 24 種水生生物之鋅濃度後，發現所有水產生物 (淡水、鹼水、海水魚類及無脊椎動物) 之鋅濃度皆在 10-100 μg/g fresh tissue 之間。但是鯉魚、鯽魚、金魚之鋅濃度卻常為其他魚之 10-20 倍以上。鯉魚及鯽魚組織之鋅濃度以消化道組織為最高，平均值在 500 μg/g fresh tissue;其他組織如腎臟、鰓、骨骼之鋅濃度亦常為 50-150 μg/g fresh tissue。至今為止，鯉魚、鯽魚、金魚內臟高濃度之鋅似乎未見於其他魚類或水生生物。

3. 鯉魚組織中高濃度鋅之特異性

(1) 其他金屬─除了鋅之外，鯉魚組織中之 Cu，Na，K，Ca 及 Mg 之濃度似乎與其他魚類沒有不同。

(2) 棲息地─鯉魚組織中之高鋅，與其棲息地似乎沒有關係，因為從高山水庫、魚塭、河川捕獲之鯉魚，皆含有高鋅。

(3) 變種─鯉魚及鯽魚 (金魚) 有許多變種─如大肚鯉、鏡鯉、革鯉、錦鯉；金魚─有 "琉金"，"黑牡丹"及 "獅頭"等變種。由實驗得知，所有不同變種之鯉魚或鯽魚 (金魚)，皆具有高濃度之鋅。

(4) 成長過程─將鯉魚經過人工孵化，由稚仔魚養至成魚；成魚再人工孵化，繼續養至第三代，由前後 7 年全部在水族箱內之養殖，得知所有鯉魚皆有高濃度之鋅。

(5) 個體差 ─ 雖然鯉魚之高鋅與上述因素無關，但是不同個體之鯉魚，其鋅濃度卻有差異。在一般狀況，鯉魚消化道組織鋅濃度最低為 ~100 μg/g fresh tissue，但最高可達 ~1,000 μg/g fresh tissue，中間值在 300-500 μg/g fresh tissue 左右。

4. 鯉魚組織中之高鋅與其生理特性

(1) 鯉魚對鋅之需求─由實驗得知，鯉魚對鋅之需求與其他魚類相近，約在 ~25 mg/kg diet。

(2) 鯉魚之高鋅，非來自食性之不同─將鯉魚與鰱魚分別人工孵化，再由稚仔魚養至成魚，皆飼以相同之人工飼料 (鋅濃度皆為~25 mg/kg diet)。結果發現，幼小或成長之鯉魚，其組織中之鋅濃度分別為鰱魚之 2 至 9 倍。既使攝食一般飼料，鯉魚仍然有甚高之組織鋅濃度。

(3) 鯉魚吸收鋅，主要來自飼料，而非來自養殖用水─由於魚類生活於水中，其組織中之鋅，可能經由消化道，或鰓進入體中。經由 ^{65}Zn 之吸收實驗 (將 ^{65}Zn 置入養殖水，或飼料中)，發現鯉魚有 95%之鋅，來自飼料；來自養殖水中者極少。另外，同樣之飼料，鯉魚由飼料中吸收之 ^{65}Zn 量為草魚、鰱

魚及吳郭魚之 3-7 倍。

(4) 鯉魚可經由高鋅飼料，累積鋅；累積之鋅可被釋出─當以高鋅飼料 (2,000 mg/kg diet) 飼養鯉魚 4 週後，鋅即累積至其消化道組織及骨骼中。經 8 週飼養後，累積在消化道組織之鋅濃度，可達 1,500 μg/g fresh tissue。但是在改換一般飼料 (~25 mg/kg diet) 2 週後，累積之鋅，可被釋放出來。即鯉魚組織中之鋅並非被 "鎖定"，而是可 "移動" 的。

5. 鯉魚之高鋅來自 43 kDa「鋅結合蛋白質」

(1) 鯉魚組織中「鋅結合蛋白質」之細胞分畫─由細胞分畫，得知鯉魚組織中之高鋅，不存在於其細胞溶液，微粒體，或粒線體中，而係存在於 ”核及細胞殘屑區分” (nuclei/cell debris fraction)中。由此實驗可知鯉魚之高鋅與水溶性之鋅─酵素，鋅─蛋白質並無關係。也與水溶性之 metallothionein 無關。

(2) 鯉魚「鋅結合蛋白質」，為一種「膜蛋白質」─利用 collagenase type IV 來分解上述鯉魚組織之 "核及細胞殘屑區分"，再經超高速蔗糖梯度分離，Zn-IMAC 及 RGD-affinity 層析，可以將鯉魚組織中高鋅由來之蛋白質分離出來。此蛋白質之分子量為 43 kDa，位於細胞膜上，為一「跨膜蛋白質」，鋅係結合於膜蛋白質在細胞外之部份。

(3) 鯉魚 43 kDa「鋅結合蛋白質」之結合特性─將含有 ^{65}Zn 之 43 kDa「鋅結合蛋白質」重組入脂質體 (liposome) 後，可研究其結合特性。由實驗得知此蛋白質與鋅之結合極強，Kd 為 0.19 μM。一個蛋白質可結合 3 個鋅分子，此結合為鋅特異性，除 Co^{2+}外，其他 10 種金屬皆不會與其競爭。此蛋白質係連接於胞外母質 (matrix) laminin 之 RGD peptide 上，其結合亦為特異性，Kd

為 4.79 μM。

6. 鯉魚 43 kDa「鋅結合蛋白質」係位於纖維母細胞 (fibroblast) 及紅血球之細胞膜上

(1) 鯉魚之高鋅與消化道結締組織之關係—由實驗得知鯉魚消化道之鋅並不存在於黏膜 (上皮組織)上，而存在於其 "去黏膜組織"。經由組織切片染色及以 43 kDa「鋅結合蛋白質」之抗體進行免疫組織化學染色後，得知「鋅結合蛋白質」係存在於結締組織之細胞中。結締組織細胞中之「纖維母細胞」之細胞膜上含有此蛋白質，此「纖維母細胞」之大小約為 ~6 μm。

(2) 鯉魚紅血球細胞膜上之高鋅—一般魚類血液中之鋅主要係存在於血漿 (plasma) (占量 50~80%) 中，但是鯉魚血液中之鋅，卻主要存在於紅血球中 (占量 ~70%)。存在於鯉魚紅血球之鋅，主要存在於其細胞膜上。鋅係結合於紅血球細胞膜之膜蛋白質外面。由 43 kDa「鋅結合蛋白質」抗體所進行之免疫螢光反應可知，鯉魚紅血球細胞膜上之鋅，亦來自 43 kDa「鋅結合蛋白質」。

7. 鯉魚之極耐缺氧與其高鋅甚有關係

如同上述，鯉魚與鯽魚在極低或完全沒有溶氧之水中，亦能存活數小時至數日。將鯉魚與鯽魚放置在缺氧之水族箱中 (溶氧為 0.00 ppm，水溫 25-31 ℃)，5 日後，發現總數 974 尾鯉魚 (10 次實驗，每次約 100 尾魚) 中，其死亡率為 17%。比較存活與因缺氧而死亡的鯉魚其鋅濃度後，發現死亡鯉魚的消化道組織鋅濃度顯著地低於存活的魚 (59±22 vs. 148±47 μg/g fresh tissue, $p<0.001$)。以鯽魚進行 6

次實驗 (每次 100 尾魚)，缺氧 5 天後發現，600 尾鯽魚中，並無魚死亡。鯽魚消化道組織中的鋅濃度為 652±458 μg/g fresh tissue，高於上述存活與因缺氧而死亡的鯉魚。利用免疫組織化學法染色後，發現死亡的鯉魚其 submucosal 層中之 43 kDa「鋅結合蛋白質」含量遠比存活的魚為少。將缺氧耐受性"較低"的鯉魚，飼以高鋅飼料 (2,000 mg/kg diet) 1~2 個月後，發現其存活率由 0%上升至 50%；若餵食 6 個月，則無魚死亡。由以上結果可知鯉魚與鯽魚對缺氧的高耐受性與組織中的高鋅及高含量的 43 kDa「鋅結合蛋白質」極為有關。

8. 鯉魚消化道組織「纖維母細胞」之分離及其初代培養

由於已知鯉魚消化道組織之高鋅，係存在於其結締組織「纖維母細胞」中，乃嘗試將其分離，並進行培養。將鯉魚消化道組織以玻片刮除其黏膜後，而得"去黏膜組織"。將鯉腸"去黏膜組織"，以 collagenase type IV 處理 6 h（30℃）後，可將"去黏膜組織"中之結締組織全部溶解，而分離出其中之細胞。此結締組織細胞由 3 類細胞組成，一為 ～6 μm 大小之細胞，占量為 ～65%；一為 ～9 μm 大小之細胞，占量為 ～30%；其他細胞占量為 ～5%。由免疫螢光及免疫酵素染色，得知～6 μm 大小之細胞為 纖維母細胞。由 Giemsa 染色及 Hematoxylin and eosin 染色得知，此纖維母細胞之細胞核占其細胞總面積之～80%，細胞質之量甚少，只有～20%。將鯉魚消化道之"去黏膜組織"切成 1 mm^3 後，置入 collagenase type IV 之 DMEM/F12 medium 中，經 20 min 後，收集"細胞溶液"。將上述"細胞溶液"在 37℃培養後，發現鯉魚之纖維母細胞在有"基底膜"存在條件下，能在 4 h 內快速生長。鯉魚之纖維母細胞與"傷口癒合"及"高度耐受缺氧"可能甚有關係。

9. 魚類之生態與生理、生化之關係

由於魚類能在極為不同之環境下也能生存，是研究其生態與生理，生化關係極為有利之生物。鯉魚由於分佈甚廣，極易飼養，亦為世界各地重要養殖魚類。魚類中同時具有①極耐不良環境 (缺氧、低溫等)②雜食③長壽等特性者，除鯉魚、鯽魚 (金魚) 外，似乎甚少。另外，具有高濃度鋅之魚類除鯉魚，鯽魚 (金魚) 之外，似乎尚未發現。鯉魚之生態特性可能與其組織中之高濃度鋅甚有關連。目前已知鯉魚之極耐缺氧與高鋅確有關連。另外當鯉魚組織受到切割後，其纖維母細胞能快速增生 (doubling time 約為 2 h)，在生物中極為少見，可能與其 "傷口癒合" 甚有關係。研究生物之適應環境，鯉魚及鯽魚 (金魚) 可能為極佳之材料。鯉魚及鯽魚之高鋅，與其環境適應，似乎甚值得深入研究。

The discovery of a novel zinc-binding protein in common carp, and its physiological meaning

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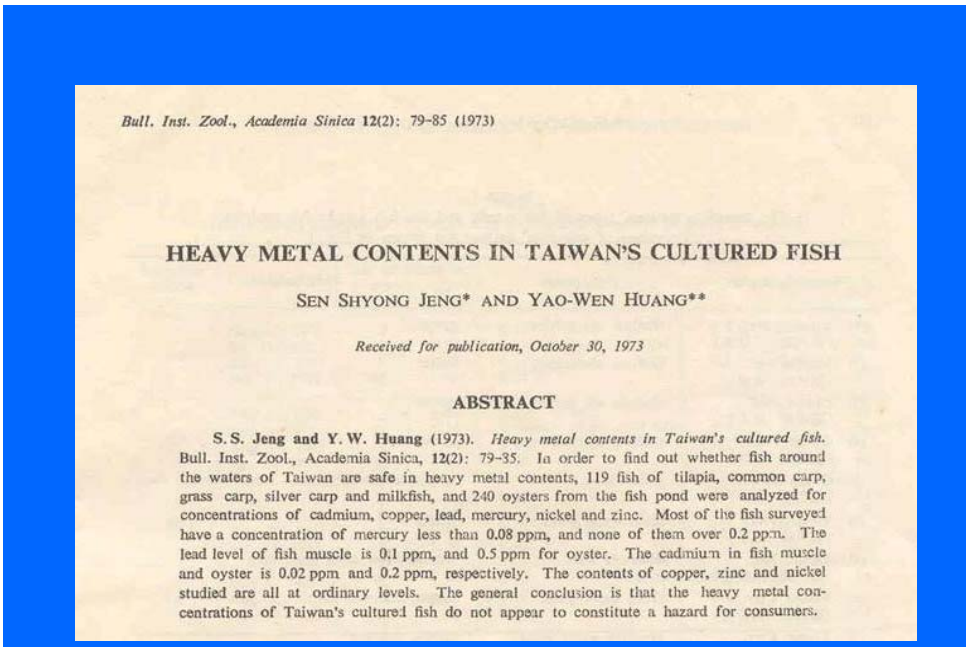
Victims of Minamata Disease (caused by Mercury polluted fish) in Japan in 1960 s



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HEAVY METAL CONTENTS IN TAIWAN'S CULTURED FISH

SEN SHYONG JENG* AND YAO-WEN HUANG**

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ABSTRACT

S. S. Jeng and Y. W. Huang (1973). *Heavy metal contents in Taiwan's cultured fish.* Bull. Inst. Zool., Academia Sinica, **12**(2): 79-35. In order to find out whether fish around the waters of Taiwan are safe in heavy metal contents, 119 fish of tilapia, common carp, grass carp, silver carp and milkfish, and 240 oysters from the fish pond were analyzed for concentrations of cadmium, copper, lead, mercury, nickel and zinc. Most of the fish surveyed have a concentration of mercury less than 0.08 ppm, and none of them over 0.2 ppm. The lead level of fish muscle is 0.1 ppm, and 0.5 ppm for oyster. The cadmium in fish muscle and oyster is 0.02 ppm and 0.2 ppm, respectively. The contents of copper, zinc and nickel studied are all at ordinary levels. The general conclusion is that the heavy metal concentrations of Taiwan's cultured fish do not appear to constitute a hazard for consumers.

Heavy metal concentrations (μg/g wet tissue) of Taiwan's cultured fish.

		Cd	Cu	Hg	Ni	Pb	Zn
Fish	muscle	0.02 (0.00-0.10)	1.4 (0.5-1.6)	0.08 (0.00-0.17)	0.3 (0.0-1.8)	0.1 (0.0-0.4)	25 (6-92)
	viscera	0.07 (0.00-0.15)	5.9 (1.4-15.0)	0.08 (0.00-0.09)	2.4 (0.1-7.2)	0.5 (0.0-2.0)	46* (16-64)
Oyster	edible protion	0.19 (0.10-0.27)	23.3 (6.6-37.9)	0.03 (0.00-0.04)	0.3 (0.1-0.6)	0.5 (0.1-0.8)	49 (23-78)

***Not including those values of common carps which had a level of 170-585 ppm.**

Specificity of the high zinc concentration in common carp and crucian carp

- **Different water habitats**
  reservoirs in high mountain, rivers, fish ponds
- **Different variants**
  Common carp – "mirror carp", "leather carp", "golden carp"
  Crucian carp,
  Gold fish – "ryukin", "telescope black moor"

All the *Cyprinus carpio* and *Carassius auratus* have high zinc concentration in their tissues

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High Zinc Concentration in Common Carp Viscera*

* コイ内臓における高濃度の亜鉛 (鄭素華・羅秀娟: 中央研究院・動物研究所, 台北, 台湾)

Table 1. Zinc concentration (μg/g wet tissue) in different tissues of common carp, *Cyprinus capio* LINNAEUS

No.	Sample: total length (cm)	Sample: total weight (g)	sex	Tissue: heart	diges-tive tract*	hepto-pan-creas	spleen	kidney	gonad	Conc. in entire viscera: without gonad	Conc. in entire viscera: with gonad
1.	24.8	235	♂	54	387	118	120	75	54	212	81
2.	23.0	194	♂	46	400	62	164	450	62	234	103
3.	23.5	217	♂	84	75	99	257	161	9	112	46
4.	22.5	224	♂	40	281	128	50	315	54	210	67
5.	26.9	302	♂	35	442	147	71	610	11	250	73
6.	25.5	233	♂	37	361	48	43	93	74	140	102
mean value				46	330	95	135	312	43	214	76

* Digestive tract without contents.

Table 2. Mean zinc concentration (μg/g wet tissue) in different tissues of grass carp, *Ctenopharyngodon idellus* CUVIER and VALENCIENNES, silver carp, *Hypophthalmichthys molitrix* CUVIER and VALENCIENNES, and tilapia, *Tilapia mossambica* PETERS

Sample*1	Tissue: heart	diges-*2 tive tract	liver	spleen	kidney	gonad	Conc. in entire viscera: without gonad	Conc. in entire viscera: with gonad
Grass carp	14	18	23	24	19	20	19	19
Silver carp	15	12	19	15	8	—	13	—
Tilapia	28	35	12	46	19	20	31	30

Zinc concentrations in some animal tissues (μ g/g fresh tissue, ppm)

Mammalian tissues (human, monkey, rat, cow, sheep)	10-100
Birds	10-100
Edible portion of finfish	3 - 24
Aquatic organisms (freshwater, brackish-water fish, marine fish, invertebrates)	10-100
Common carp, crucian carp	
digestive tract tissue	562±287
kidney	191±112
spleen	134± 70
skeletal tissues	139± 56
muscle	34± 18
Eyes, spermatozoa	300-5,000

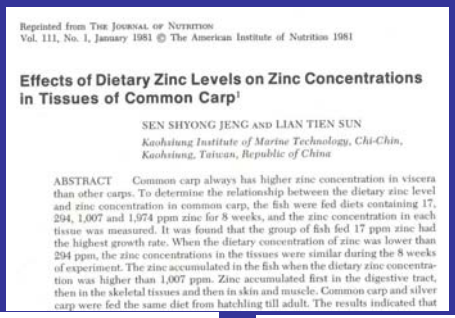
Common carp
cyprinus carpio

Crucian carp
Carassius auratus

Ecology of common carp and crucian carp

- **Worldwide distribution, wide range of fresh water habitats**
- **Optimum water temperature 25~30℃, but could live between 4~37℃**
- **Tolerate to hypoxia (dissolved oxygen 1~2 ppm) for several weeks**
- **Tolerate to anoxia (dissolved oxygen 0 ppm) for several days**
- **Feeds on detritus and live food on the bottom**
- **longevity—always>50 years old, 150 years olds?**

High zinc in common carp and its physiological properties



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Effects of Dietary Zinc Levels on Zinc Concentrations in Tissues of Common Carp[1]

SEN SHYONG JENG AND LIAN TIEN SUN

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ABSTRACT Common carp always has higher zinc concentration in viscera than other carps. To determine the relationship between the dietary zinc level and zinc concentration in common carp, the fish were fed diets containing 17, 294, 1,007 and 1,974 ppm zinc for 8 weeks, and the zinc concentration in each tissue was measured. It was found that the group of fish fed 17 ppm zinc had the highest growth rate. When the dietary concentration of zinc was lower than 294 ppm, the zinc concentrations in the tissues were similar during the 8 weeks of experiment. The zinc accumulated in the fish when the dietary zinc concentration was higher than 1,007 ppm. Zinc accumulated first in the digestive tract, then in the skeletal tissues and then in skin and muscle. Common carp and silver carp were fed the same diet from hatchling till adult. The results indicated that

Zoological Studies 33(1): 78-85 (1994)

Zoological Studies

Comparison of Zinc Absorption between Common Carp and Other Fresh Water Fishes

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(Accepted July 6, 1993)

Sen-Shyong Jeng and Juang-Lin Lian (1994) Comparison of zinc absorption between common carp and other fresh water fishes. *Zoological Studies* **33**(1): 78-85. In order to survey absorption rate differences between common carp and other fresh water fishes, ^{65}Zn was added to diet or water and zinc absorption studied. Results indicate that common carp absorb 3 times more zinc from their diet than tilapia, 3.4 times more than grass carp, and 6.8 times more than silver carp. The relative absorption rates of ^{65}Zn from water among common carp and other fishes were found to be insignificant. Obviously the reason why common carp have a higher zinc concentration in its tissue than other fishes is due to that it has several times higher absorption rate of zinc from diet than other fishes. Under experimental conditions, common carp absorbed 86% of its zinc from diet, whereas, tilapia, grass carp and silver carp absorbed 86%, 78%, and 78% of their zinc from diet, respectively. Diet was the predominate source for zinc absorption in all fishes studied. The absorption channel of zinc from water in common carp was investigated. It was found that gill was the major channel.

Key words: Zinc, Carp, Zinc absorption.

Fish Physiology and Biochemistry **20**: 313-324, 1999.
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313

Accumulation of zinc from diet and its release in common carp

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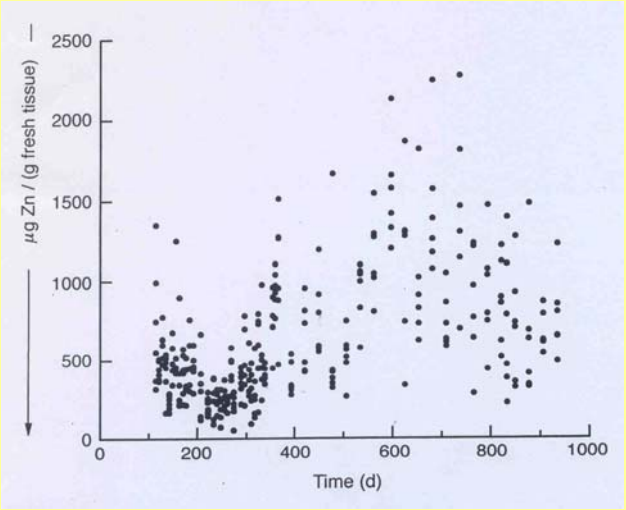
Accepted: December 4, 1998

Key words: digestive tract tissue, distribution of zinc, grass carp, metallothionein, release of zinc, subcellular, tilapia

Abstract

High zinc diet of 2000 mg kg^{-1} was fed to common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*) and tilapia (*Oreochromis aureus*) for 8 weeks to compare the accumulation of Zn in fish. It was found that accumulation of zinc from diet by grass carp and tilapia was low when compared to common carp. The largest difference occurred in digestive tract tissue. The digestive tract tissue of common carp had a large pool of stored zinc, the mean 'storable capacity' is about 1500 $\mu g\ Zn\ g^{-1}$ fresh tissue, and the time needed to saturate the tissue when feeding 2000 mg $Zn\ kg^{-1}$ diet is about 8 weeks. The accumulated Zn in the digestive tract tissue of common carp was released when the dietary Zn was reduced to normal level (50 mg kg^{-1}) or deficient level (4 mg kg^{-1}) for 4 weeks.

Zinc concentration in common carp from fry to adult fish (d 0 to d 930, F1-F3)



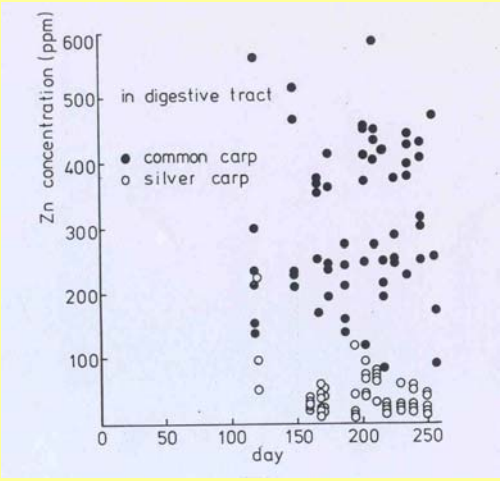
Cu, Na, K, Ca, Mg of the common carp are in the same levels as in other fishes

Zn, Cu, Na, K, Ca, and Mg concentrations (μg/[g fresh tissue]) in digestive tract tissues of common carp, grass carp, silver carp, and tilapia

Fish	Zn	Cu	Na	K	Ca	Mg
Common carp	454±214	3±1	1416± 96	3203±126	42±10	134±12
Grass carp	18± 1	4±1	1596±158	2976± 91	47±11	140±18
Silver carp	18± 6	2±1	1292±131	3070±209	25± 6	175±46
Tilapia	25± 3	4±1	2086± 62	2946±196	74± 3	133± 4

mean±SD, n=6

High zinc in common carp was not caused by the diet

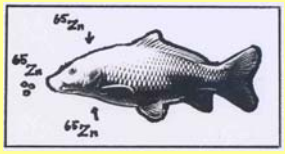


Zinc concentrations in common carp and silver carp fed the same diet (~25 mg/kg diet) for 250 d

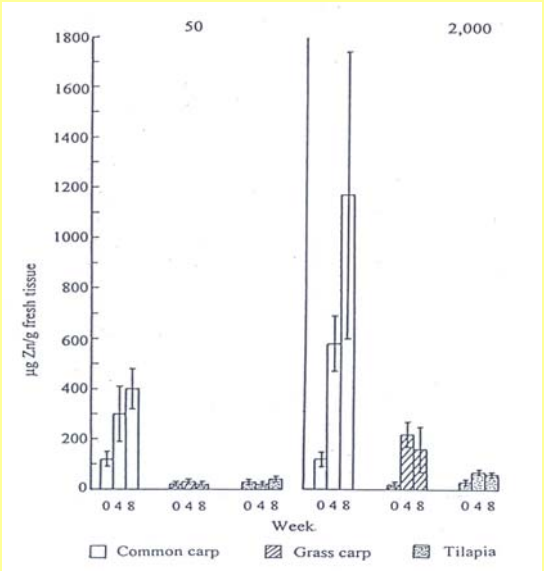
Common carp absorbs zinc from diet, but not from " culturing water"

Relative absorption rates of Zn between diet and water in common carp and other fresh water fishes

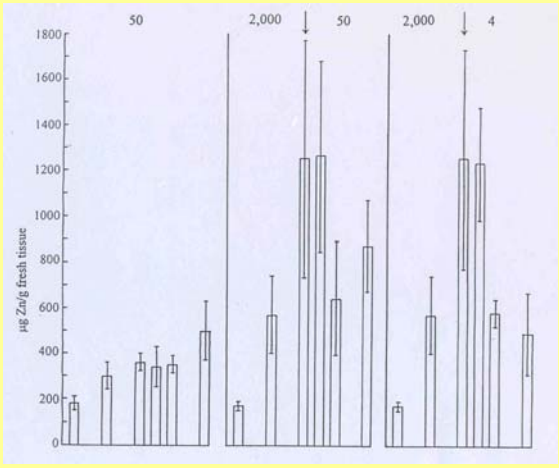
Fish	Amount of Zn absorbed from diet (μg/g fish)	Amount of Zn absorbed from water (μg/g fish)	Relative absorption rate of Zn between diet and water diet : water
Common carp	0.230	0.014	95: 5
Tilapia	0.068	0.012	85:15
Grass carp	0.065	0.018	78:22
Silver carp	0.040	0.011	78:22



Common carp accumulate zinc from diet, but other fishes did not



Zinc accumulated in common carp could be released (Digestive tract tissue of common carp is a zinc reservoir)



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High zinc in common carp and its biochemical properties

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Comparative Biochemistry and Physiology Part B 122 (1999) 461–468

CBP

Zinc and zinc binding substances in the tissues of common carp

Sen-Shyong Jeng *, Jih-Terng Wang, Lian-Tien Sun

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Received 15 June 1998; received in revised form 4 February 1999; accepted 10 February 1999

Abstract

Extraordinarily high concentrations of zinc (200–500 μg/g fresh tissue) are often found in the digestive tract tissue of common carp *Cyprinus carpio*, and high zinc concentrations (typically > 100 μg/g fresh tissue) are also found in the kidney, gill, skeletal tissues, and spleen. In the present study, we found that only about 40% of the zinc in the digestive tract tissue of common carp could be extracted by water. However, 0.01 M citrate buffer, pH 4.2 could extract over 90% of the zinc. Subcellular zinc distribution in the tissues of common carp, grass carp *Ctenopharyngodon idellus*, silver carp *Aristichthys nobilis*, and tilapia *Oreochromis aureus* were compared. It was found that zinc concentrations in the cytosol, microsomal and mitochondrial fractions were approximately the same for all four species, being only about 16, 5, and 4 μg/g fresh tissue), respectively. However, zinc concentrations in the nuclei/cell debris fraction of common carp tissue were much higher (40–330 μg/g fresh tissue) than the < 14 μg/g fresh tissue) found in the other three species. From this we conclude that neither water-soluble zinc proteins nor metallothionein could account for the high levels of zinc found in common carp tissues. A preliminary biochemical investigation suggests that the main zinc binding substance(s) in the nuclei/cell debris fraction of digestive tract tissue of common carp was probably a membrane protein(s). © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Common carp; Zinc; Digestive tract tissue; Subcellular distribution of zinc; Zinc binding substance; Zinc binding protein; Metallothionein; Membrane protein

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Biochemical and Biophysical Research Communications 309 (2003) 733–742

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Isolation of a Zn-binding protein mediating cell adhesion from common carp☆

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Department of Food Science, College of Life Science and Resources, National Taiwan Ocean University, Keelung 202, Taiwan

Received 10 August 2003

Abstract

Extraordinarily high concentrations of Zn (300–500 μg/g fresh tissue) are often found in the digestive tract tissue of common carp *Cyprinus carpio*, and most of the Zn is bound to membrane protein located on plasma membranes that are attached to basal laminae. To isolate the Zn-binding protein, the basolateral plasma membranes were separated from the extracellular matrix by treating the nuclei/cell debris fraction of the tissue with collagenase type IV and Arg-Gly-Asp (RGD) peptide. The Zn-binding protein was isolated from the separated plasma membranes by immobilized metal affinity chromatography and affinity chromatography on laminin-Sepharose. A 43 kDa protein was bound by the laminin-Sepharose and specifically eluted with tri-elbin (a mimic of RGD). Affinity chromatography on wheat germ agglutinin and concanavalin A-Sepharose showed that the 43 kDa protein is a glycoprotein. The 43 kDa protein was labelled with ^{65}Zn and became incorporated into liposomes at a high efficiency. Liposomes containing this protein were bound to laminin-Sepharose or reconstituted basement membrane. We propose that the Zn-binding protein is a cell surface receptor involved in the adhesion of cells to laminin.

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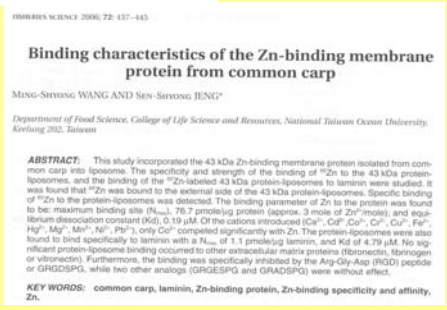
Keywords: Zn; Zn-binding protein; Common carp; RGD peptide; Cell adhesion; Laminin; Extracellular matrix



FISHERIES SCIENCE 2006; 72: 437–443

Binding characteristics of the Zn-binding membrane protein from common carp

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Department of Food Science, College of Life Science and Resources, National Taiwan Ocean University, Keelung 202, Taiwan

ABSTRACT: This study incorporated the 43 kDa Zn-binding membrane protein isolated from common carp into liposome. The specificity and strength of the binding of ^{65}Zn to the 43 kDa protein-liposomes, and the binding of the ^{65}Zn-labeled 43 kDa protein-liposomes to laminin were studied. It was found that ^{65}Zn was bound to the external side of the 43 kDa protein-liposomes. Specific binding of ^{65}Zn to the protein-liposomes was detected. The binding parameter of Zn to the protein was found to be: maximum binding site (N_{max}), 76.7 pmole/μg protein (approx. 3 mole of Zn^{2+}/mole); and equilibrium dissociation constant (Kd), 0.19 μM. Of the cations introduced (Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}), only Co^{2+} competed significantly with Zn. The protein-liposomes were also found to bind specifically to laminin with a N_{max} of 1.1 pmole/μg laminin, and Kd of 4.79 μM. No significant protein-liposome binding occurred to other extracellular matrix proteins (fibronectin, fibrinogen or vitronectin). Furthermore, the binding was specifically inhibited by the Arg-Gly-Asp (RGD) peptide or GRGDSPG, while two other analogs (GRGESPG and GRADSPG) were without effect.

KEY WORDS: common carp, laminin, Zn-binding protein, Zn-binding specificity and affinity, Zn.

High zinc in common carp is not water soluble zinc-enzyme, zinc-protein, or metallothionein

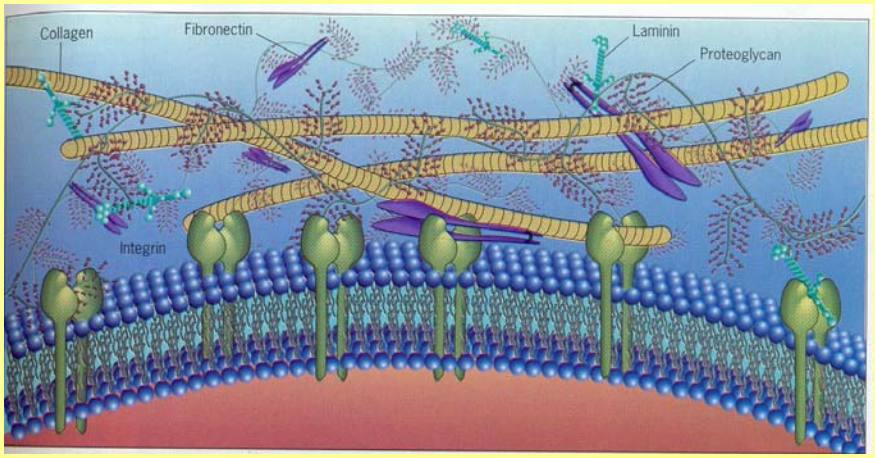
Extraction of Zn in digestive tract tissue of common carp with different aqueous solutions

Solvent	pH	Conc. (M)	No. of samples	% of Zn extracted
H_2O			4	36± 6
NaCl		0.1	11	38± 6
Sodium acetate-acetic acid buffer	4.0	0.2	1	67
Phosphate buffer	7.1	0.02	1	40
KH_2PO_4-NaOH buffer	7.4	0.05	6	62±21
Tris-HC1 buffer	8.4	0.05	10	45± 4
Glycine-NaOH buffer	9.0	0.05	3	59±17

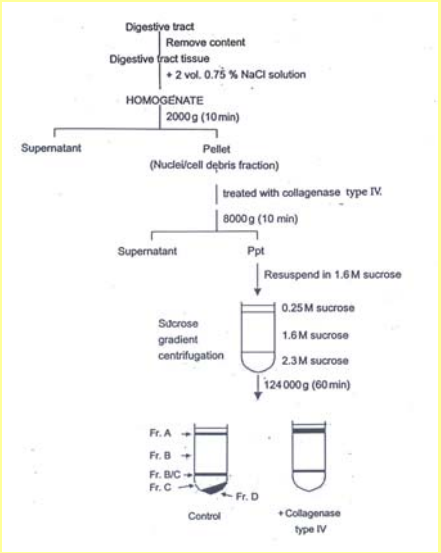
Subcellular fractionation indicated that the high zinc in common carp existed in nuclei/cell debris fraction

Table 4 Zn concentrations (μg/[g fresh tissue]) in subcellular fractions of some tissues of common carp, grass carp, silver carp and tilapia.

Tissue	Zn conc. in whole tissue	Zn conc. in subcellular fraction				
		Cytosol fraction[a]		Microsome fraction	Mitochondria /lysosome fraction	Nuclei/cell debris fraction
		HMF	LMF			
1. Common carp (n=16)[b]						
Digestive tract tissue	449±248[c]	20± 8	42±32	10±5	7±4	370±218
Kidney	249±112	19± 8	2± 1	5±3	3±1	218±106
Hepatopancreas	81± 27	23±10	2± 1	4±3	6±5	46± 19
Spleen	74± 24	10± 7	0	3±2	7±5	53± 20
2. Grass carp (n=6)						
Digestive tract tissue	28± 9	13± 8	1± 1	3±1	2±1	7± 4
3. Silver carp (n=6)						
Digestive tract tissue	26± 9	15± 8	0	2±2	3±1	9± 7
4. Tilapia (n=6)						
Digestive tract tissue	42± 2	14± 2	5± 2	6±1	3±1	14± 1



An overview of the macromolecular organization of the extracellular matrix

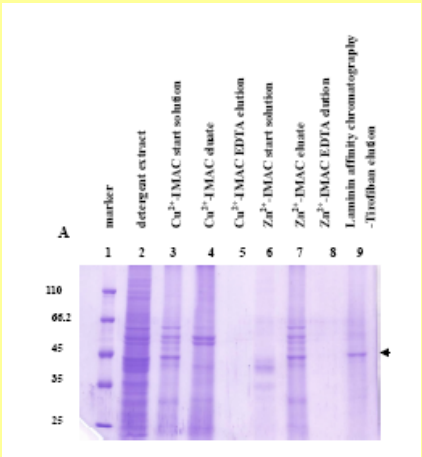


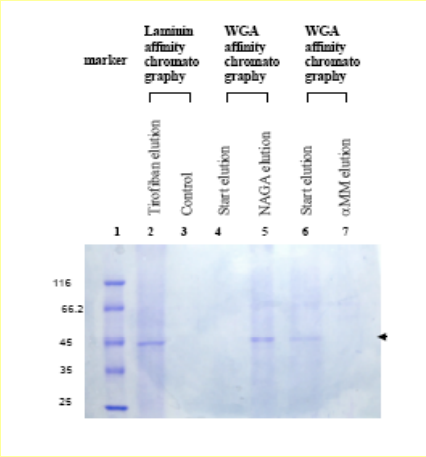
Isolation of the zinc-binding protein-I

a.Cellular subfractionation

b.Treated with collagenase type IV

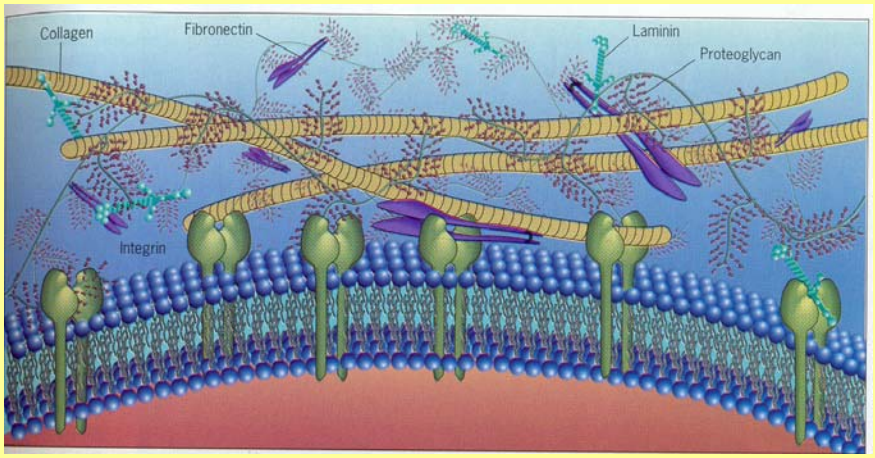
c.Sucrose gradient centrifugation





Isolation of the zinc-binding protein-II

a.Detergent extraction
b.Cu-imobilizized affinity chromatography
c.Zn-imobilizized affinity chromatography
d.Laminin affinity chromatography
e.Lectin affinity chromatography

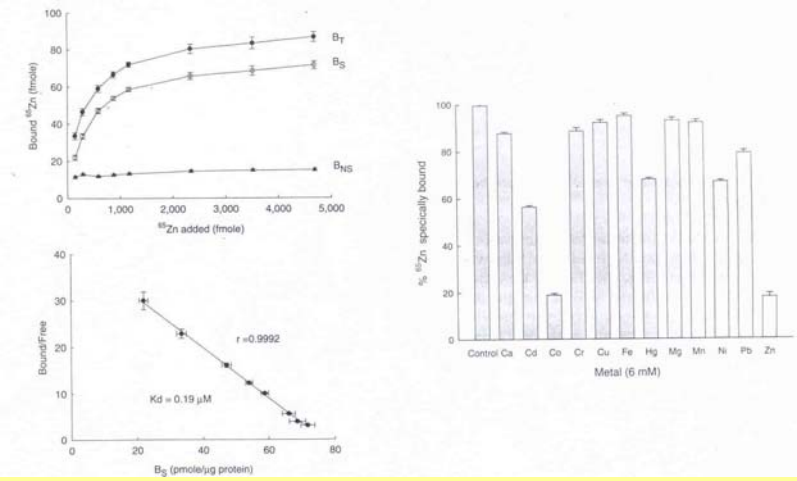


An overview of the macromolecular organization of the extracellular matrix

(The zinc-binding protein of common carp has a molecular weight of 43 kDa which appears to be a glycoprotein on cell surface mediated cell adhesion to laminin of collagen type IV)

Binding characteristics of the 43 kDa Zinc-binding protein

- Binding of zinc: 3 mole of Zn^{2+}/mole protein
- Kd＝0.19 μm
- No binding competition with Zn^{++} from other metals, except Co^{2+}



What kind of cell in common carp which holds the 43 kDa zinc-binding membrane protein?

FISHERIES SCIECNE 2002; 68: 484-493

Localization of Zn-binding protein in the digestive tract tissue of common carp

MING-SHYONG WANG,[1] PEI-CHI CHUANG,[1] LIAN-TIEN SUN[2] AND SEN-SHYONG JENG[1]*

[1]*Department of Food Science and* [2]*Institute of Marine Biology, College of Life Science and Resources, National Taiwan Ocean University, Keelung 202, Taiwan*

ABSTRACT: The concentrations of Zn and sulfhydryl (SH) groups in the digestive tract tissue of common carp and some aquatic animals were studied. It was found that Zn and bound SH groups could be used as indicators for detecting the Zn-binding protein in the digestive tract tissue of common carp. The digestive tract tissue of the fish underwent subcellular fractionation, and it was found that the nuclei/cell debris fraction contained most of the DNA (85%), Na^+/K^+-ATPase (82%), organic phosphate (90%) and the Zn-binding protein (79%), but only part of the 5'-nucleotidase and alkaline phosphatase (<23%). The nuclei/cell debris fraction of the digestive tract tissue of common carp was treated with either collagenase type I or type IV, and subfractionated by sucrose density centrifugation. It was found that treatment with collagenase type IV could release more than 50% of the Zn-binding protein, Na^+/K^+-ATPase and organic phosphate from collagen. Sections of digestive tract tissue of common carp were stained for Zn. It was observed that Zn can be found mainly on the edge of the epithelial layer, and everywhere in the 'membrane-like' portion of the submucosal and muscular layers. It is proposed that most of the Zn-binding protein in the digestive tract tissue of common carp is located on the basolateral plasma membranes of the epithelial cells and on the surrounding muscle cells that are attached to the collagen type IV of basal laminae.

KEY WORDS: basal lamina, basolateral plasma membranes, collagenase, common carp, localization of zinc, sulfhydryl groups, zinc-binding protein, zinc.

FISHERIES SCIECNE 2006; 72: 893-902

Association of zinc with connective tissue in the digestive tract of common carp

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Department of Food Science, College of Life Sciences, National Taiwan Ocean University, Keelung 202, Taiwan

ABSTRACT: Zinc (Zn) concentration in the digestive tract of common carp is always >10 times higher than most animal tissues. In a previous paper, it was reported that this high Zn came from a 43 kDa Zn-binding membrane protein. In this present study it was further found that in the digestive tract of common carp, Zn content was closely associated with the amounts of extracellular macromolecules. The higher the Zn content, the more there is of collagen and glycosaminoglycans. An indirect immunoperoxidase staining method using antibody against the 43 kDa Zn-binding protein, or the fibroblast marker (Thy 1.1 protein) was applied to the sections of digestive tract of fish. It was found that the Zn-binding protein in the digestive tract of common carp is mainly located in the connective tissue of its lamina propria and submucosal layer. Connective tissue cells, probably fibroblasts, hold the 43 kDa Zn-binding protein. In the common carp Zn might be bound to the external side of the fibroblast. The present finding may have a significant meaning on extending the studies of Zn in biology to the field of Zn with extracellular matrix.

KEY WORDS: common carp, connective tissue, digestive tract, extracellular matrix, fibroblast, Zn-binding protein, Zn.

FISHERIES SCIECNE , Vol 73 (2) 2007

High Zn in the erythrocyte plasma membranes of common carp *Cyprinus carpio*

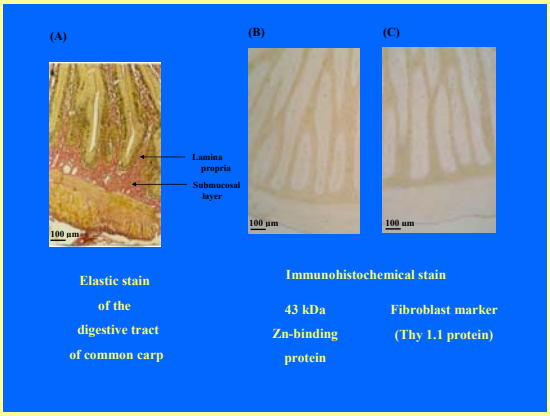
Sen-Shyong Jeng*, Jeng-Yeng Yau, Yen-Hua Chen Tzu-Yung Lin and Yueh-Ying Chung

Department of Food Science, College of Life Sciences, National Taiwan Ocean University, Keelung 202, Taiwan

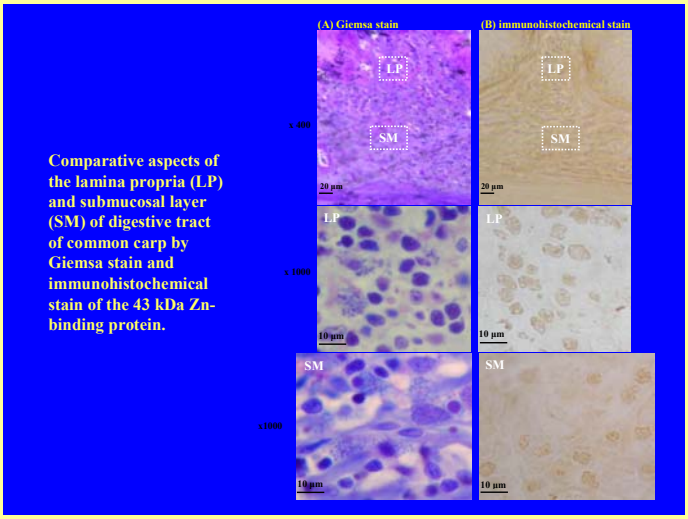
ABSTRACT: The distribution of Zn concentration in the blood and erythrocytes of common carp, grass carp, silver carp and tilapia was studied. It was found that in whole blood, the average Zn concentrations in these four species of fish (~6 to ~14 μg/[ml whole blood]) were relatively similar to those in other species of fish and mammals. However, the mean Zn concentration in the erythrocytes of common carp is about 2 times higher than the other 3 species of fish (~5 vs. ~2 μg/[ml whole blood]). It was found that ~70% of the Zn in the common carp whole blood came from its erythrocytes. In addition, ~43% of the Zn in the erythrocytes of common carp was found to be located on its outer plasma membranes. When an antibody against a 43 kDa Zn-binding protein isolated from the digestive tract tissue of common carp was used, significant quantities of the protein were shown to be present on the erythrocyte plasma membranes of common carp by an indirect immunofluorescent staining. High Zn on the outer plasma membrane in common carp is most probably comes from the 43 kDa Zn-binding protein.

KEY WORDS: Zinc, Zn-binding protein, blood, erythrocytes, biomembranes, common carp

The zinc-binding protein exists in the connective tissue (lamina propria, submucosal layer) of the digestive tract of common carp



The zinc-binding protein exists in fibroblast of the connective tissue of the digestive tract of common carp



FISHERIES SCIENCE 2008; 74: 1322–1329

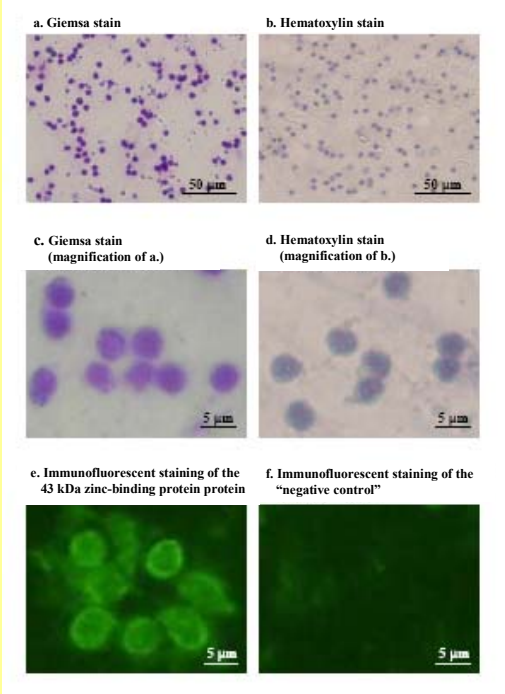
Separation and characterization of connective tissue cells expressing 43-kDa zinc-binding protein from common carp

YEN HUA CHEN, HSUAN JUNG LIAO AND SEN SHYONG JENG*

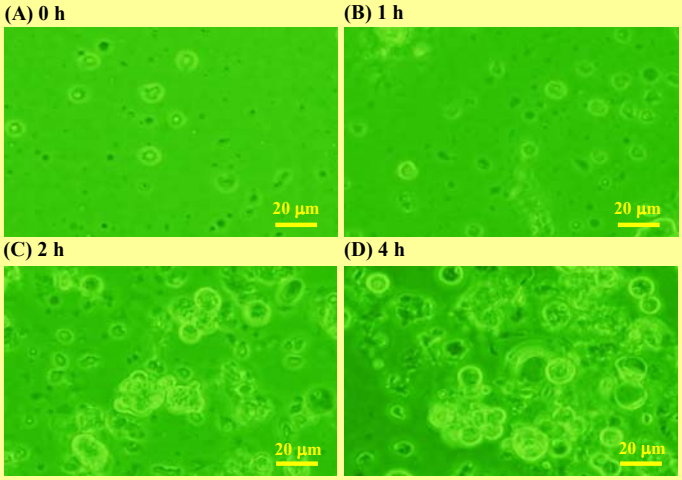
Department of Food Science, College of Life Sciences, National Taiwan Ocean University, Keelung 202, Taiwan

ABSTRACT: To separate the connective tissue cells expressing the 43-kDa zinc-binding protein from the common carp, mucosa of the digestive tract of the fish was removed by scraping with a glass slide, and the de-mucosa tissue was digested with a collagenase type IV solution. The cells collected from the collagenase-treated suspension were rather homogeneous, and more than 90% of the cells were round with a diameter of approximately 6 μm. Significant quantities of the 43 kDa zinc-binding protein were shown to be present on the cell surface of the approximately 6-μm cells. The mean zinc concentration in the cells was found to be 2.21 μg zinc/10^6 cells, which is approximately 20–30 times higher than that found in the other three fish species (grass carp, silver carp, tilapia) studied. The present work may provide a basis for the culture of these cells.

KEY WORDS: **common carp, connective tissue cells, fish cells, zinc-binding protein, zinc.**

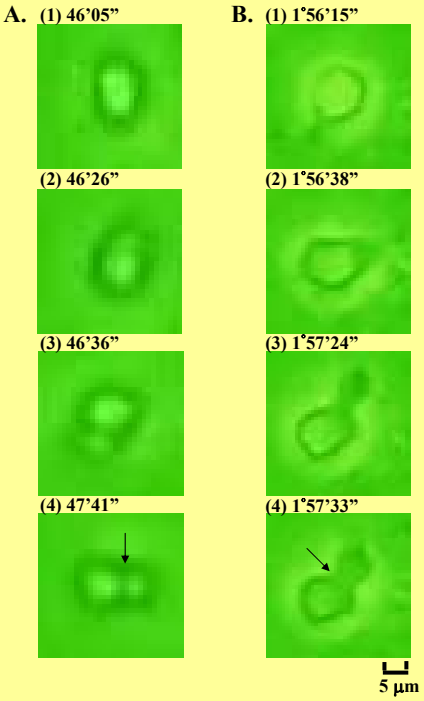


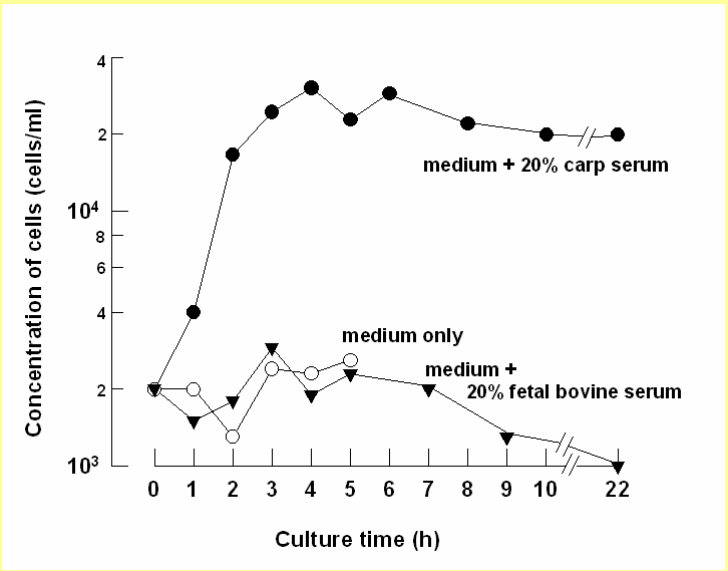
Cell culture of the connective tissue cells from common carp



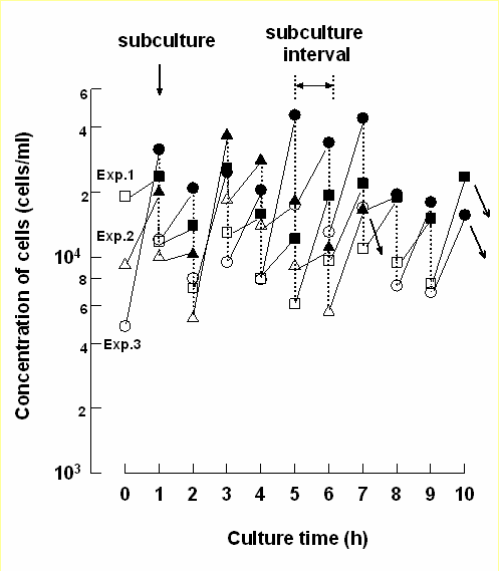
Primary explant culture of the connective tissue cells from common carp in 4 h (phase-contrast micrographs)

Sequential phase-contrast micrographs of the mitotic figure of a connective tissue cell



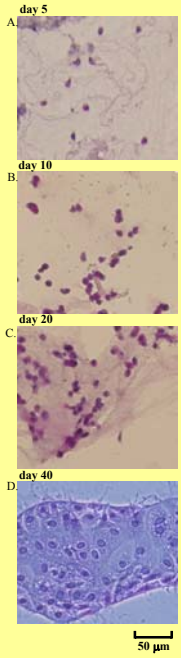


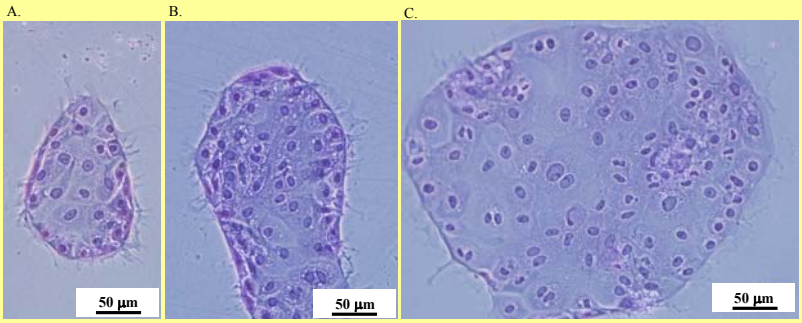
Growth curve of the connective tissue cells from common carp



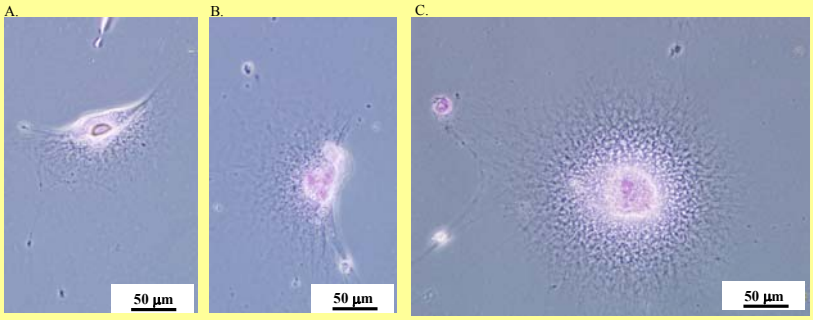
Serial subculture of the connective tissue cells from common carp in suspension.

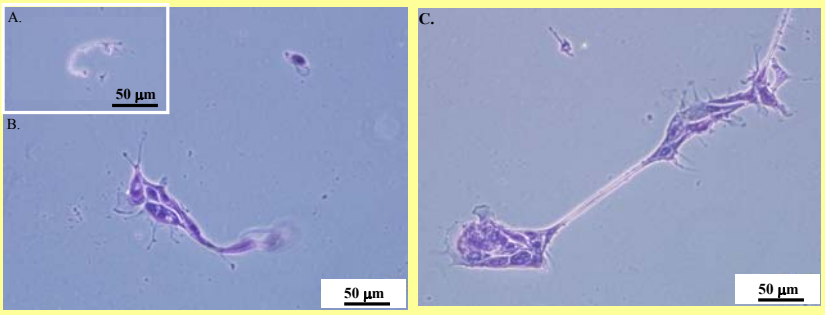
Growth of the connective tissue cells from common carp at d 5, d 10, d 20 and d 40 (Periodic acid Schiff and Hematoxylin stain).





Periodic acid Schiff and Hematoxylin stain of the connective tissue cells adhered to the culture wells of d 40 of the primary explant culture.


A.
50 µm
B.
50 µm
C.
50 µm


A.
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Anoxia survival in common carp and crucian carp is related to high zinc concentration in tissues

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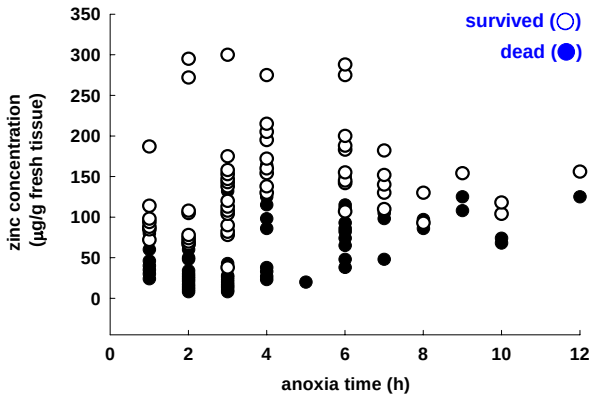
ABSTRACT: Ten lots of common carp and six lots of crucian carp (each lot of 100 fish) were treated under anoxia for five days at water temperatures of 25–31°C. The average per group mortality of common carp was 17%, but none of the 600 crucian carp died. The digestive tract tissues of the common carp that died had significantly lower zinc concentrations than those that survived (59 ± 41 *vs* 142 ± 60 μg/[g fresh tissue], $P < 0.001$). The digestive tract tissues of the crucian carp had mean zinc concentrations of 652 ± 458 μg/(g fresh tissue). One lot of common carp that had low tolerance for anoxia was fed a high zinc diet (2000 mg zinc/kg diet) for 1, 2 or 6 months and then subjected to 5 days anoxia. The survival rates of those fed the high zinc diet 1 and 2 months increased from 0 to 50%, respectively; all of fish that had fed a high zinc diet for 6 months survived. Thus, anoxia survival in common carp and crucian carp is closely related to the high concentrations of zinc in their tissues.

KEY WORDS: **anoxia, *Carassius carassius*, common carp, crucian carp, *Cyprinus carpio*, zinc.**

Common carp could tolerate anoxia (D0, 0 ppm) for several days

Mortality and zinc concentration in common carp under 5 day anoxia.

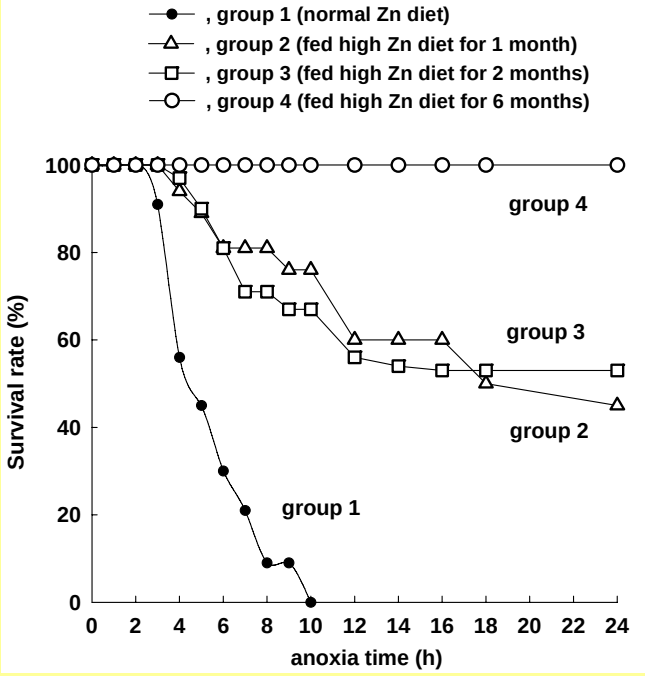
Experi-ment	Fish size		Fish dead /total fish number	Mortality (%)	Zn concentration in digestive tract tissue (μg/g fresh tissue)	
	total length (cm)	total weight (g)			dead fish	survived fish
1	13±1	36±12	0/100	0		117± 51 (n=12)
2	16±1	57±11	0/100	0		142± 71 (n=12)
3	16±2	72±39	0/100	0		119± 66 (n=12)
4	12±1	23± 2	1/100	1	38 (n=1)	128± 35 (n= 7)***
5	16±1	50± 8	5/100	5	40±29 (n=5)	139± 32 (n= 7)***
6	14±2	22± 5	9/100	9	27±13 (n=9)	244±138 (n= 9)***
7	18±1	71±15	10/100	10	122±33 (n=5)	233± 77 (n= 5)***
8	15±1	42±11	20/ 84	24	97±27 (n=20)	146± 30 (n=20)***
9	15±1	43±14	27/ 90	30	34±18 (n=27)	99± 27 (n=21)***
10	14±2	31±11	94/ 94	100	127±40 (n=94)	

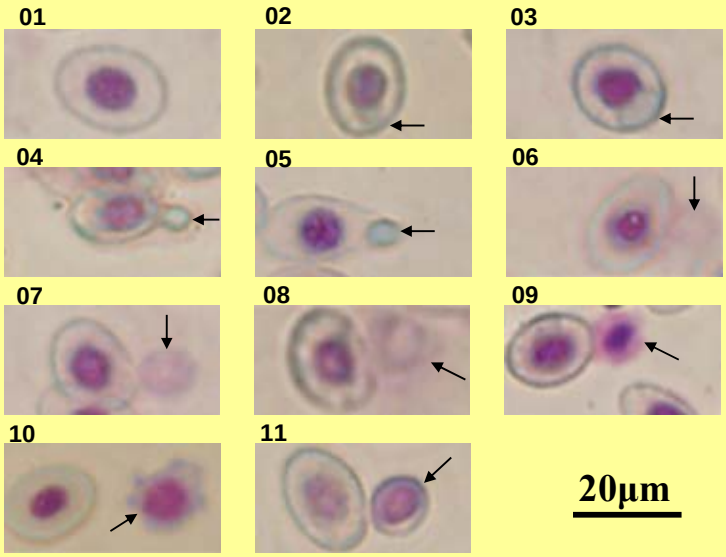


Zinc concentration in the survived (○) and dead (●) common carp digestive tract tissue under anoxia.

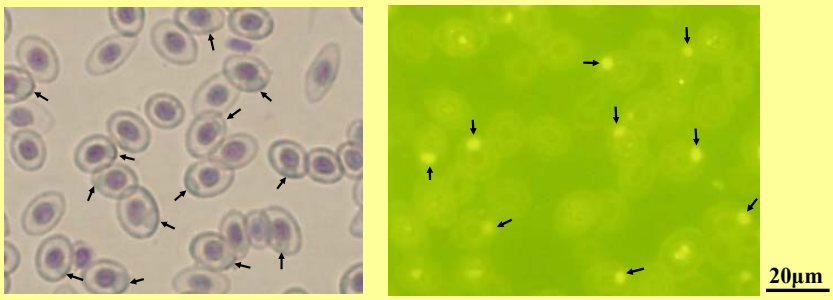
Zinc concentration in the digestive tract tissue of common carp after feeding high zinc diet for different month.

Group	Fish size		Zn concentration (μg/g fresh tissue)		
	total length (cm)	total weight (g)	whole tissue	After subcellular fractionation: water soluble (cytosol and others) fraction	After subcellular fractionation: water insoluble (nuclei/cell debris) fraction
1. basal diet	14±2	31±11	127± 40	44± 21	83± 28
2. high Zn diet for 1 month	13±1	22± 5	247± 74	99± 22	148± 55
3. high Zn diet for 2 months	12±1	24± 8	251± 38	71± 19	180± 30
4. high Zn diet for 6 months	18±2	87±28	2645±1022	782±314	2022±842





Proliferation of Red Blood Cell in common carp after being treated with cortisol or under anoxia



Giemsa stain

Immunofluorescent stain of the zinc-binding protein

Relationship between Ecology of Fish with their Physiological and Biochemical properties

- **Fish as Model Systems (Powers, 1989)**
  Fish represent the largest and most diverse group of vertebrates. Elucidating the array of mechanisms that animals use to adapt to diverse environments is one of the exciting challenges for modern biology; fish provide excellent models to meet that challenge.

- **Common carp could adapt to many enviromental stress (low dissolved oxygen, wide range of water temperature, …) and has a long life —— an excellent experimental animal.**

Ecology and Physiology of Common carp and the Zinc-binding protein

—— Further Research ——

1. **Anoxia-survival is associated with the zinc-binding protein —— Mechanism?**
2. **Rapid growth of the fibroblast (population doubling time only 1 h) —— Relationship with wound-healing?**
3. **Relationship between the high zinc in common carp and those in animal eyes, spermatozoa?**
4. **Survival in low water temperature (4℃), sudden temperature drop —— Related to the zinc-binding protein?**