

Preparative new technology for high quality frozen fish as “sashimi” and its principle

-Suppressive effect of ATP on denaturation of fish muscle protein during frozen-



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2015,10,28 APEC meeting at Kagoshima

Background of research

- “WASHOKU” added to UNESCO Intangible Heritage.
- “Sashimi” is the main dish in the traditional Japanese cuisine.
- The culture of eating of raw fish is globally spreading.



Raw fish: fresh or frozen treated?
Parasites issue

Regulation

- 1) EU : Freezing requirements for fishery products intended to be eaten raw or lightly cooked
- 2) NYC: New York City requires restaurants to freeze raw fish before serving

How effect dose “frozen and thawing” on fish meat?

Quality issues

Denaturation of myofibril protein

- Softness (low texture)
- Drip

Oxidation of myoglobin

- Discoloration

Solution ?

- Deep freezing storage ▪ ▪ High cost

Changes in amount of export and import countries of Yellowtail

平成24年農林水産物・食品輸出実績

(品目別)

食料産業局輸出促進グループ



平成25年4月

農林水産省

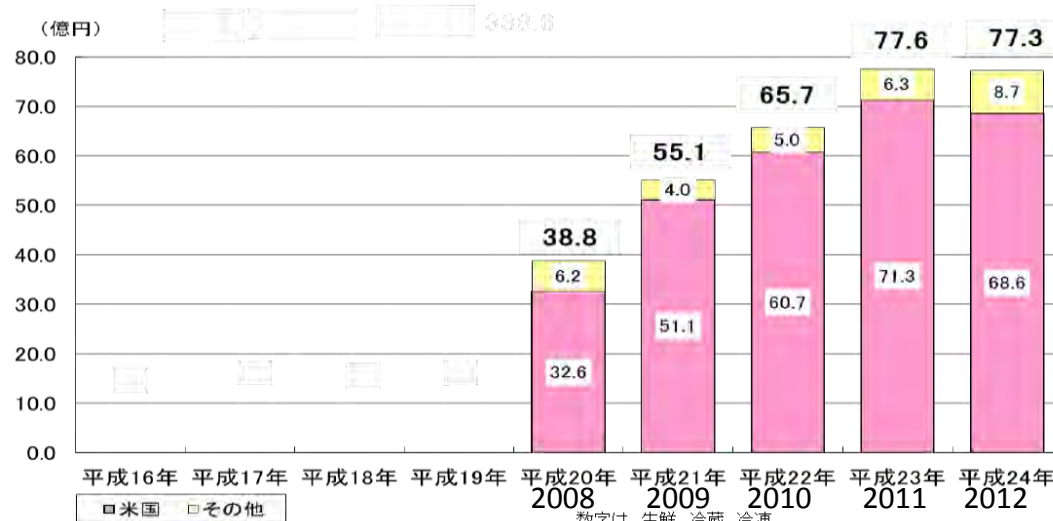


ぶりの輸出額・輸出先国の推移

MAFF

- ぶりの輸出額は、米国向けを中心に順調に増加。

The amount of export of yellowtail to USA increased



数字は、生鮮・冷蔵・冷凍
ぶり(生・蔵・凍)は2008年から分類
資料:財務省「貿易統計」を基に農林水産省作成

Export and distribution of yellowtail

[Technical issues]

- Color of dark muscle changes to brown from fresh red during frozen storage at -20°C.
- The color of **CO-treated** fish muscle does not change during frozen storage. USA permits CO-treatment. (transport by frozen)
- EU, Japan, Australia, China etc. ban the CO-treatment of fish. (transport by chilled → air transportation = high cost)
- We have to develop new technology instead of CO-treatment.
Our research target!

- Color of dark muscle changes to brown from fresh red during frozen storage at -20°C .

Why amberjack could not be transported and stored by frozen at around -20°C . This storage condition causes rapid progression of autoxidation of myoglobin and browning of dark muscle, reducing the commercial value of fish for sashimi.

Amberjack fillet

quick-frozen at -40°C

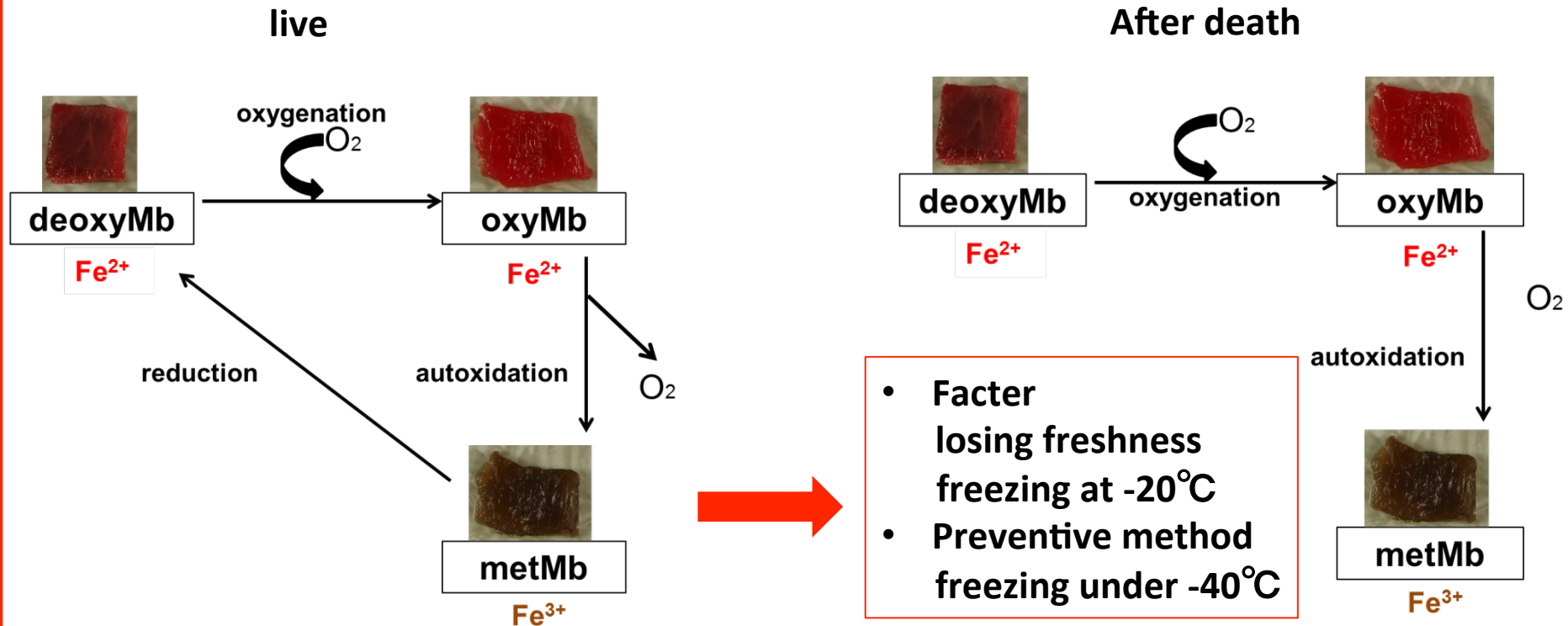


Stored for a month at -20°C



Background

Change of fish Mb.



Research objectives

Development of a technology for suppression of autoxidation of fish oxymyoglobin to metmyoglobin stored at $-20^{\circ}C$ without CO-treatment

Background Function of ATP

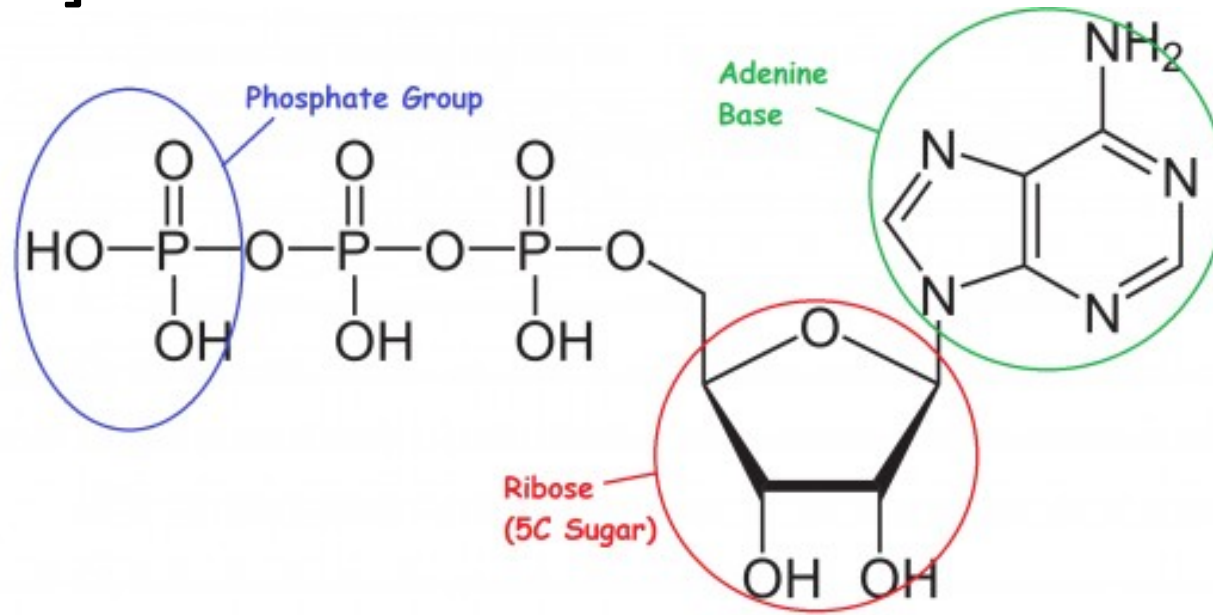
Suppression effect of ATP on the denaturation of fish Mf protein .

- ① Suppressing effect of ATP on freeze denaturation of fish myofibrilla protein
OGATA et al. Bull Japan Soc Sci Fish 2012;**78**:461-467
- ② Stabilization of squid myosin subfragment-1 and myofibrils
YOSHIOKA et al. *Fish.Sci.*2002;**68**:222-226
- ③ Re-examination of Protective Effect of ATP on Thermal Inactivation of Myosin Ca-ATPase
YOSHIOKA et al. Bull Japan Soc Sci Fish 1991;**57** :143-147



Does ATP prevent the discoloration of tuna Mb?

[ATP]

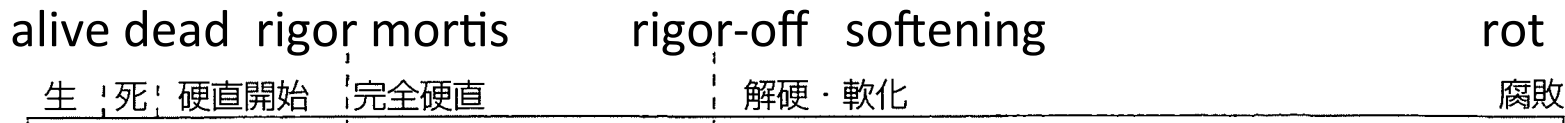


ATP = energy currency of life

Concentration in muscle: 8-10 mM

Changes in muscle state after death

(渡部)



自己消化

Self digestion

細菌による分解

Decomposition by bacteria

“生き”の限界(ATP消失)

生食の限界 (Marginal quality of sashimi grade)

marginal point of alive (ATP=0)

Degree of rigor mortis

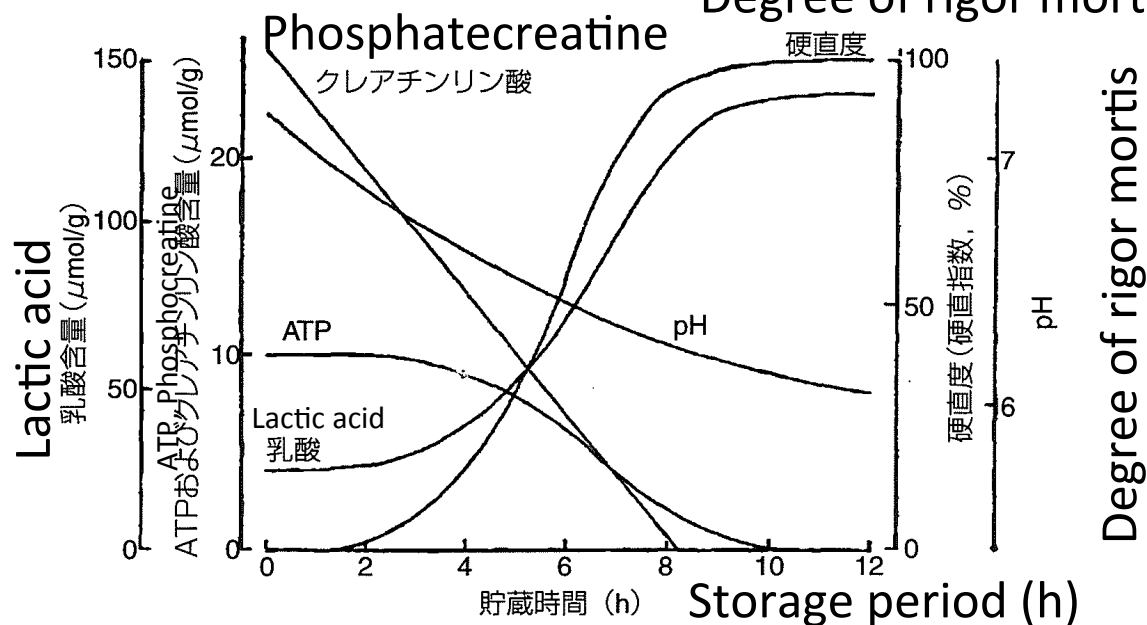


図5.1 魚の死後変化の概要³⁾

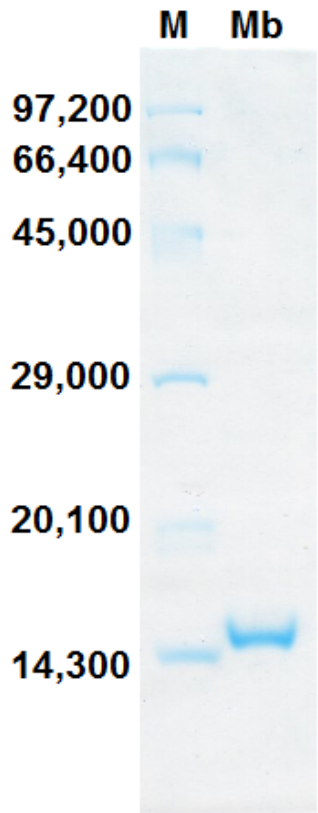
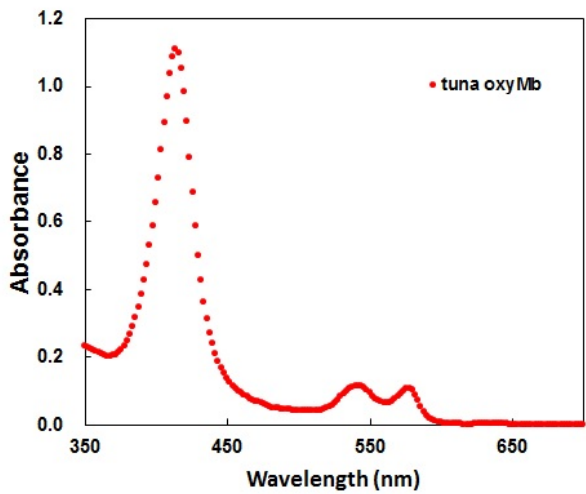
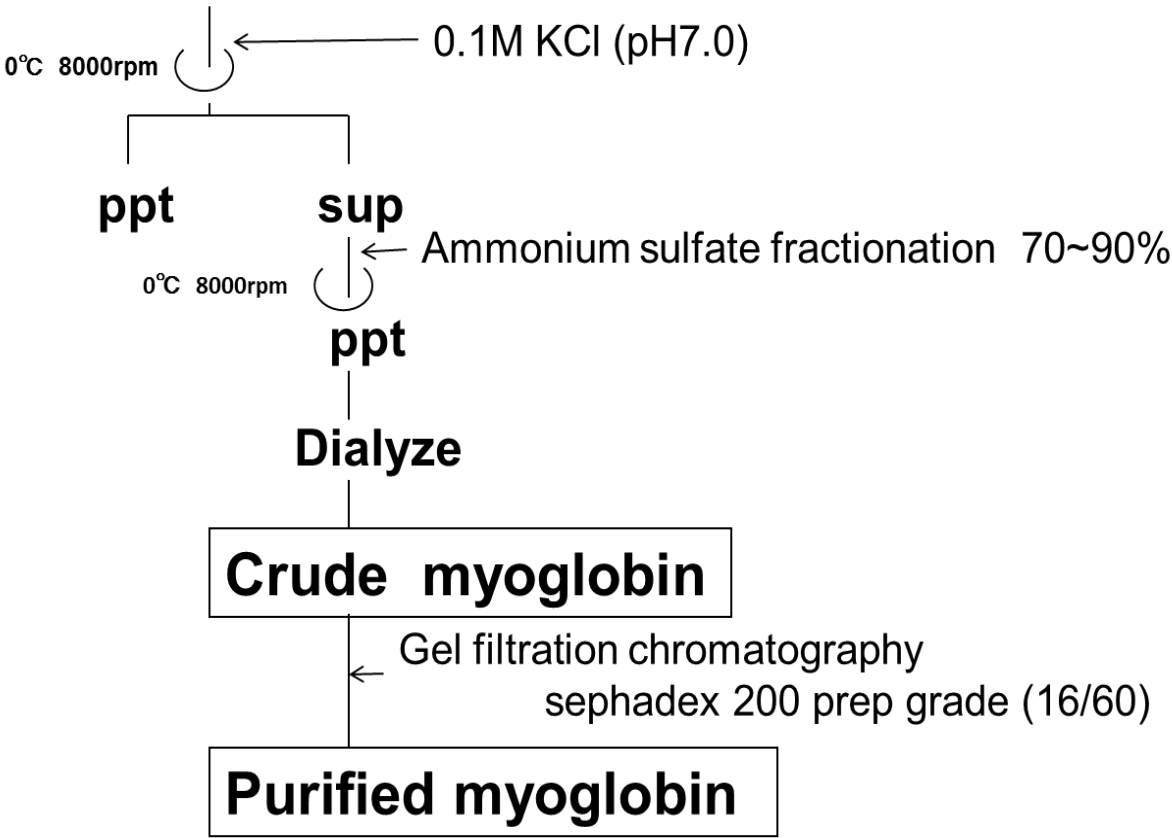
Changes in muscle state after death

Preparation of southern bluefin tuna Mb

sample

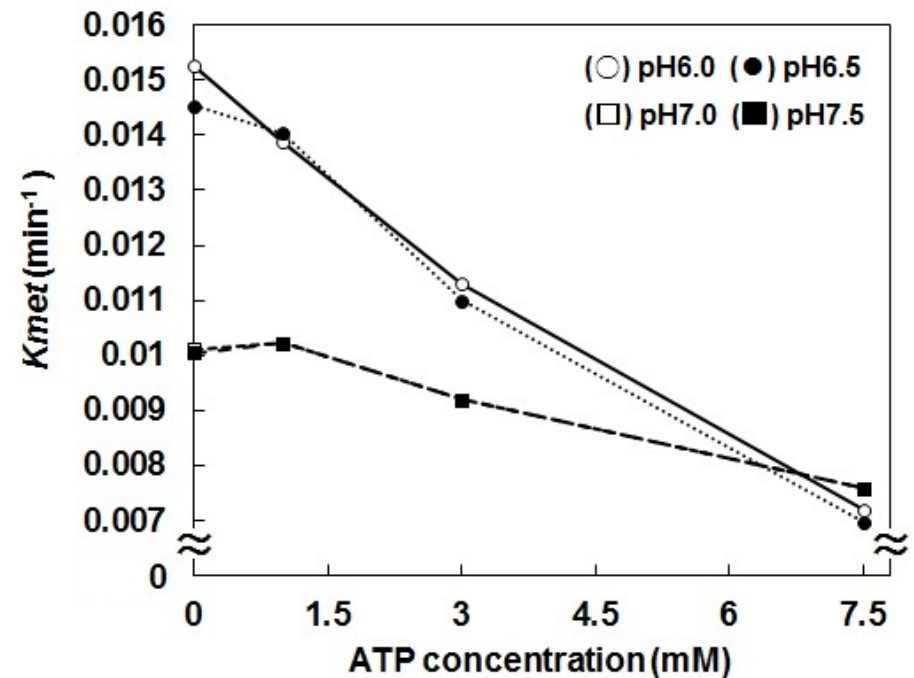
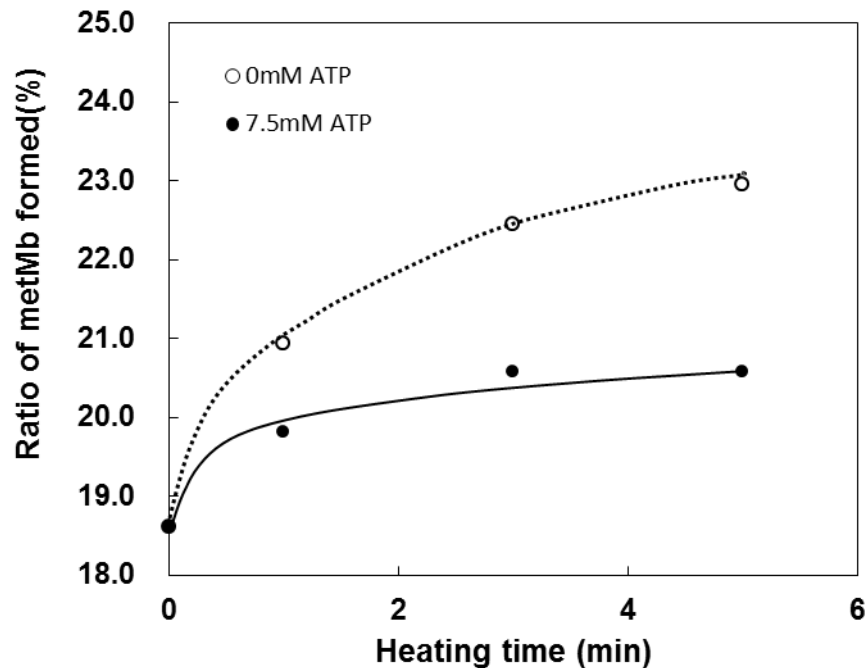
Highly freshness tuna stored at -80°C

Southern bluefin tuna meat



Effect of ATP and pH on the autoxidation rate (K_{met}) of tuna Mb at 25°C.

Effect of ATP pH6.0



We found suppressive effect of ATP on autoxidation of tuna oxymyoglobin to metmyoglobin.

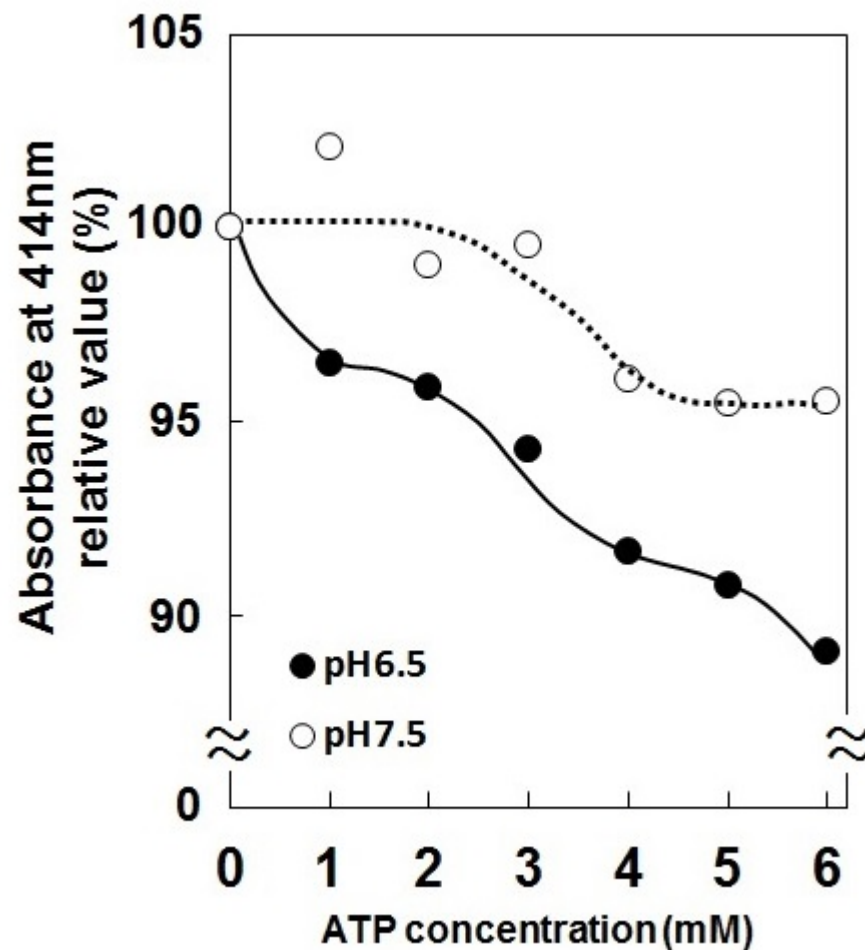
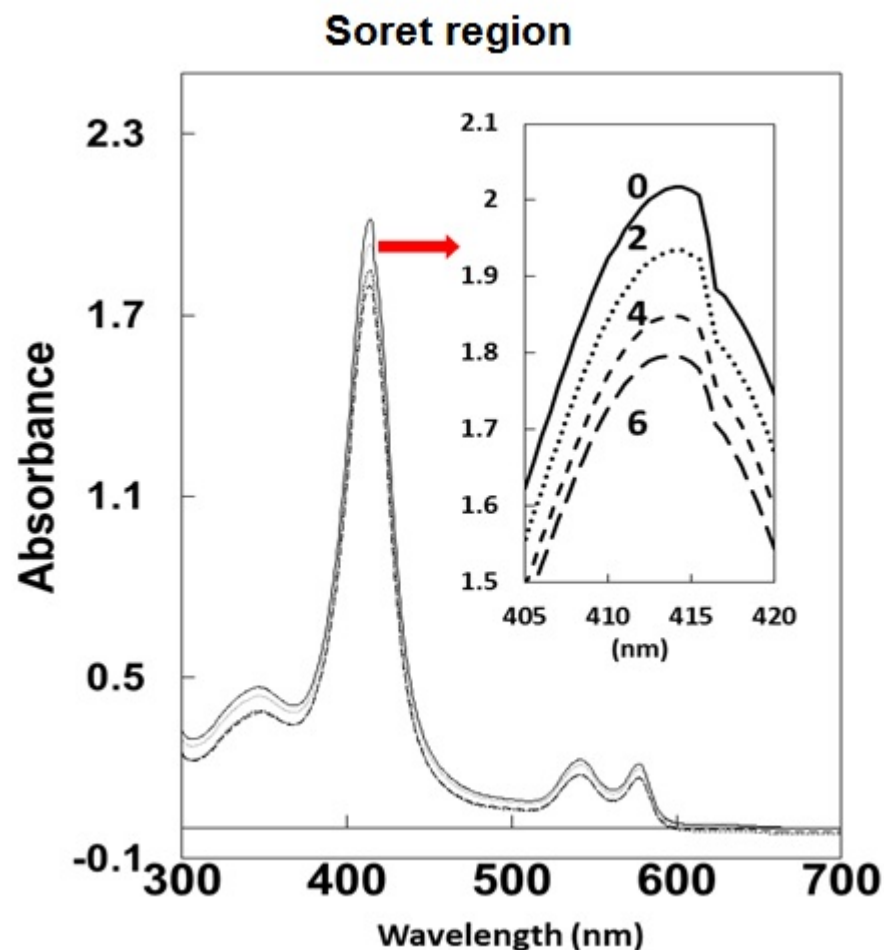
What mechanism of suppressive effect of ATP on autoxidation of Mb

How effect of ATP on Mb structure

- **Visible absorbance spectrum**
- **Circular dichroism spectrum**
- **Fluorescence spectrum**
- **Analysis of molecular size in solution and protein charge**

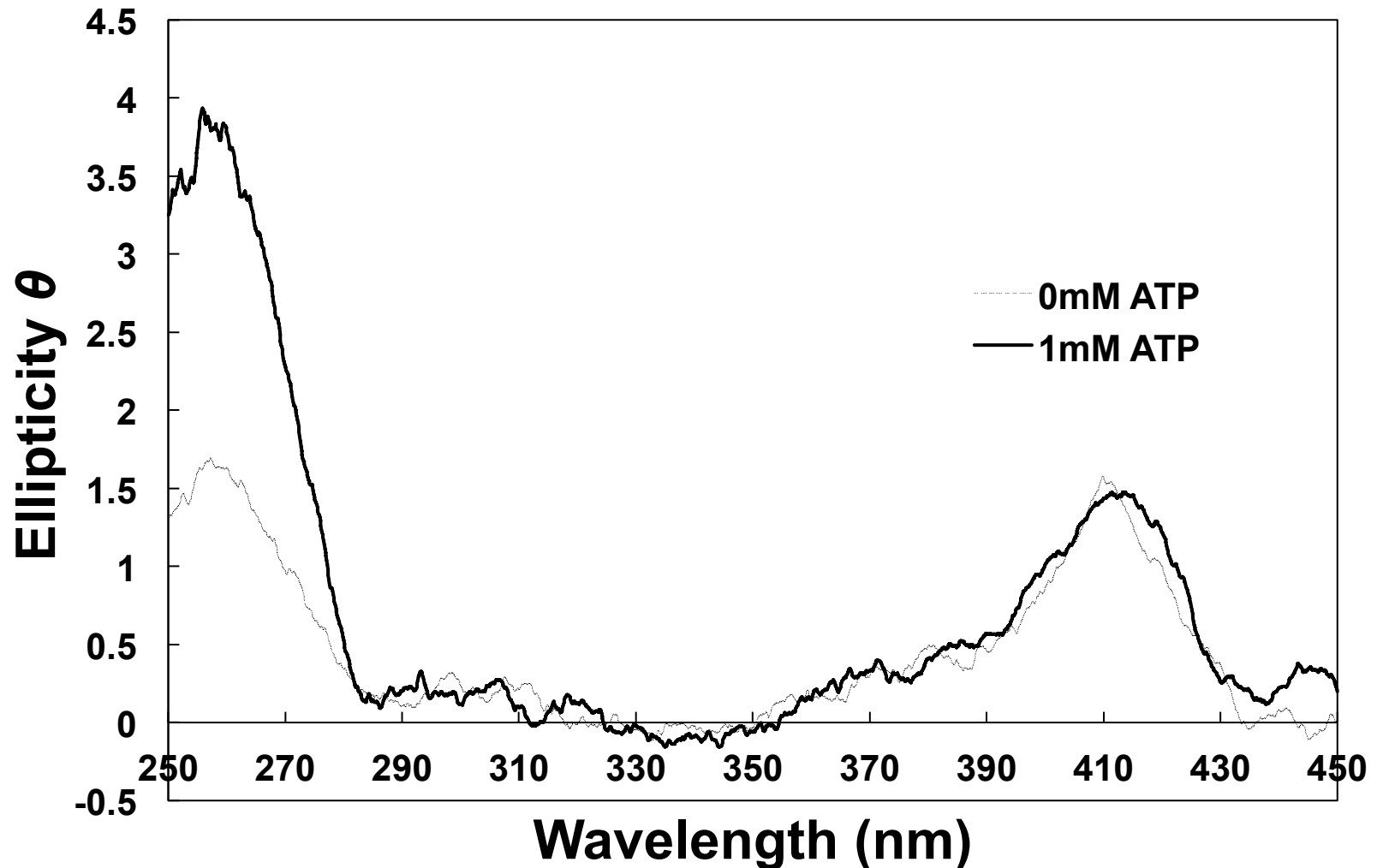
Effect of ATP on the Soret region (380-440 nm) and the visible spectra of Mb

Condition 0.1M KCl, 20mM Tris bufer (pH6.5, 7.5), Mb 0.5mg/ml, 5°C

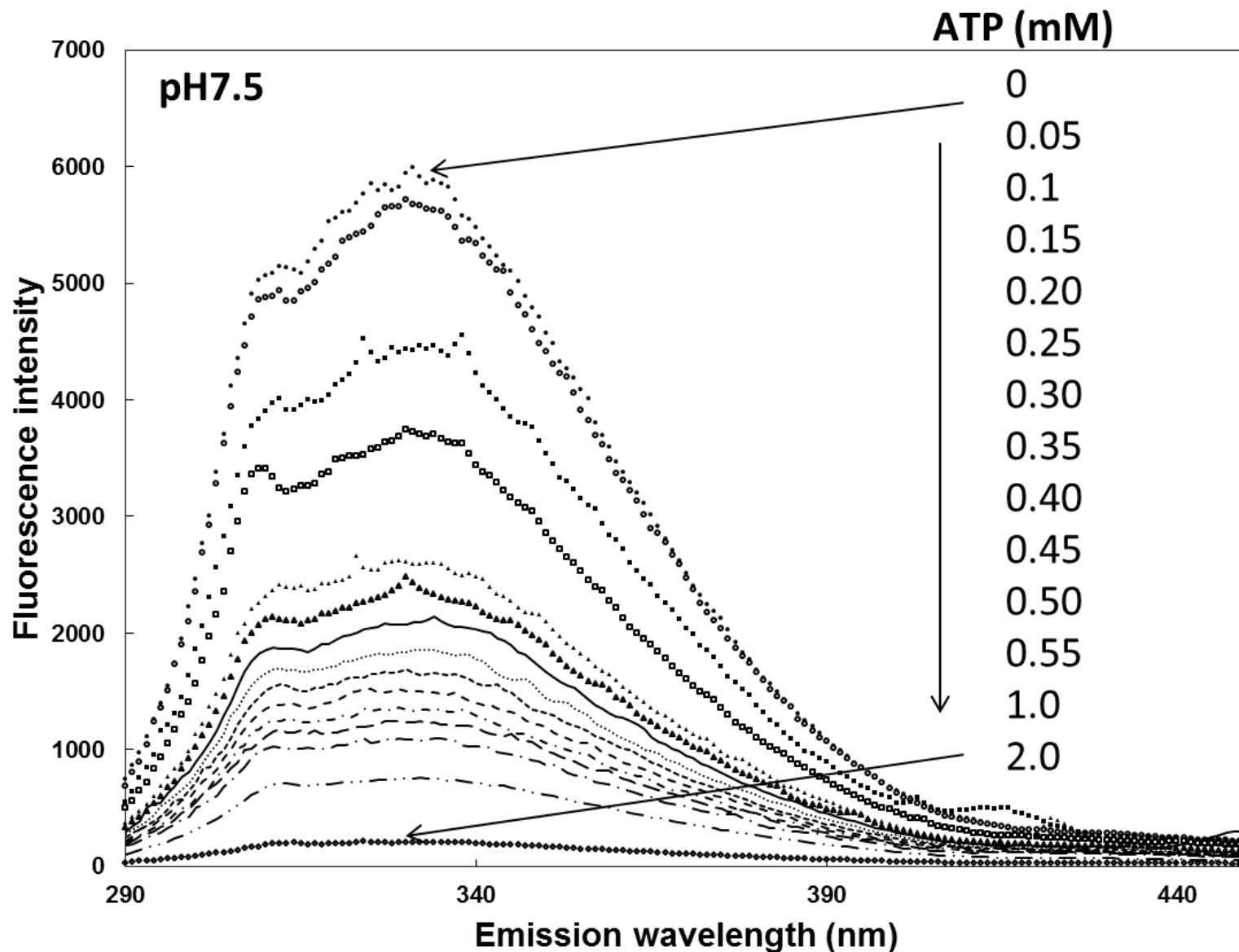


ATP-induced changes in CD spectra of native Mb

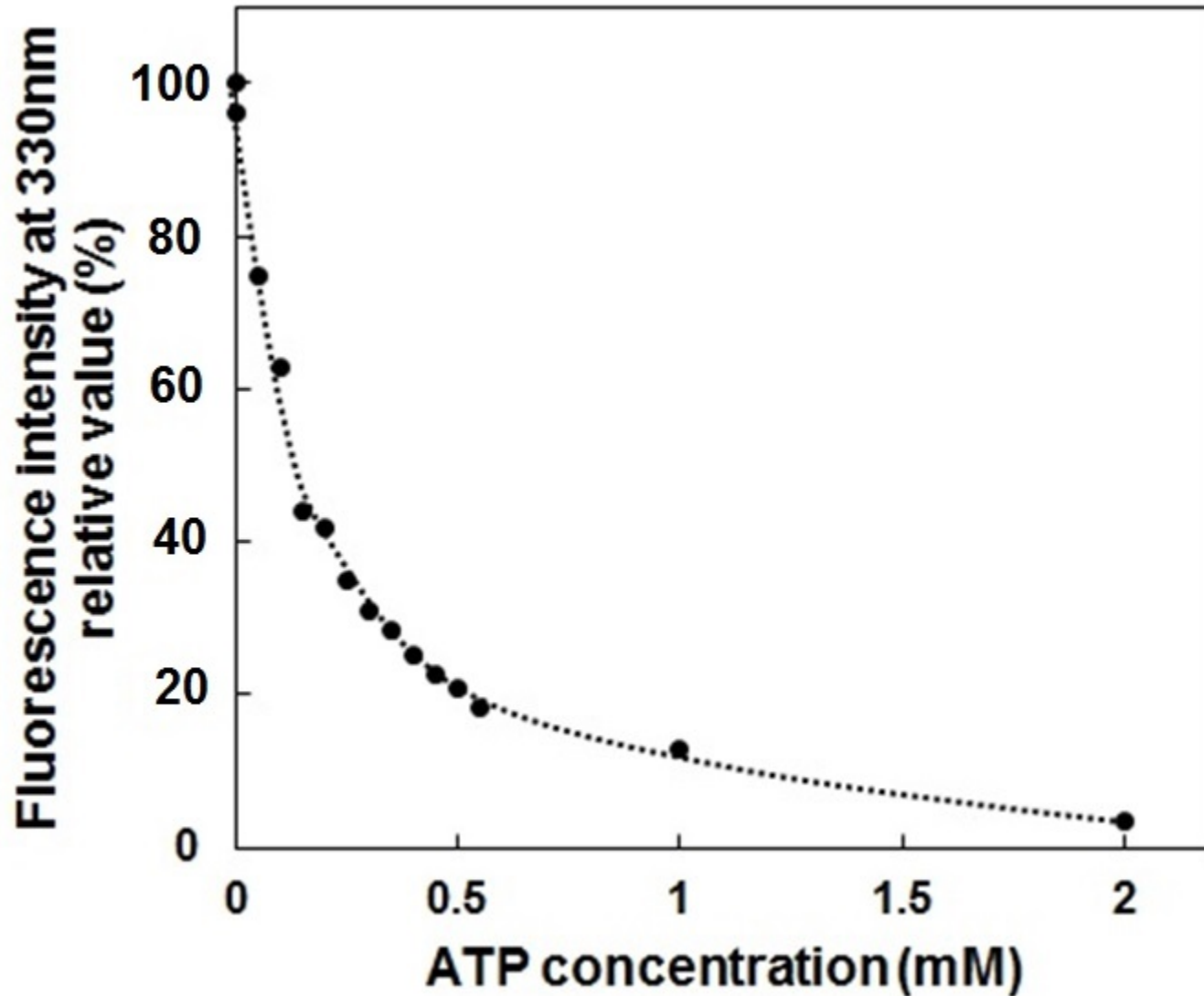
Condition 0.1M KCl, 20mM Tris-HCl pH7.5, Mb (0.7mg/ml), 10°C



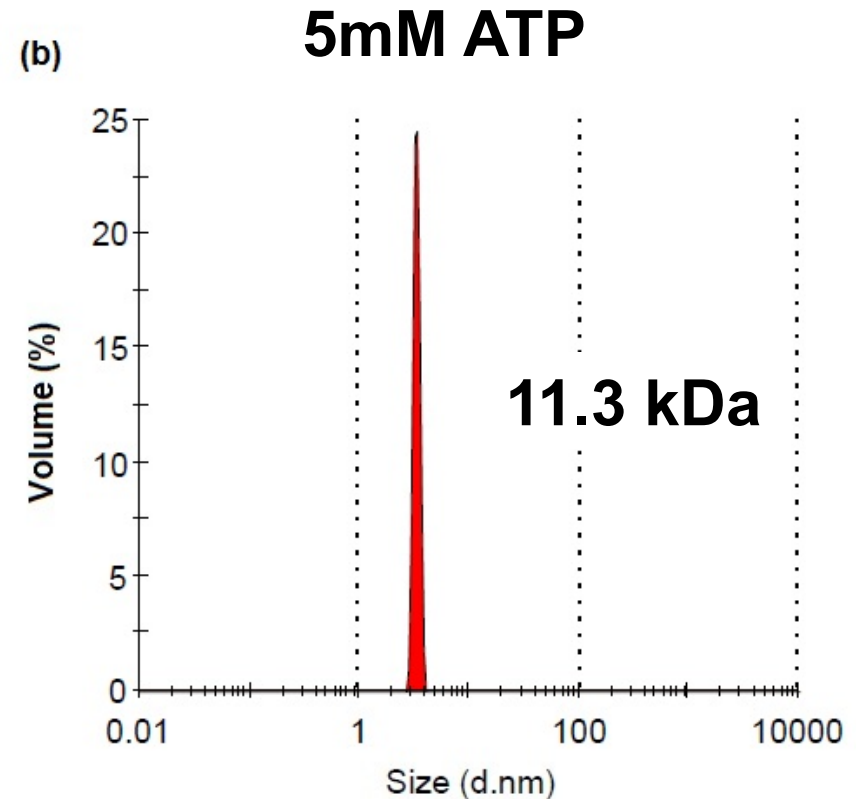
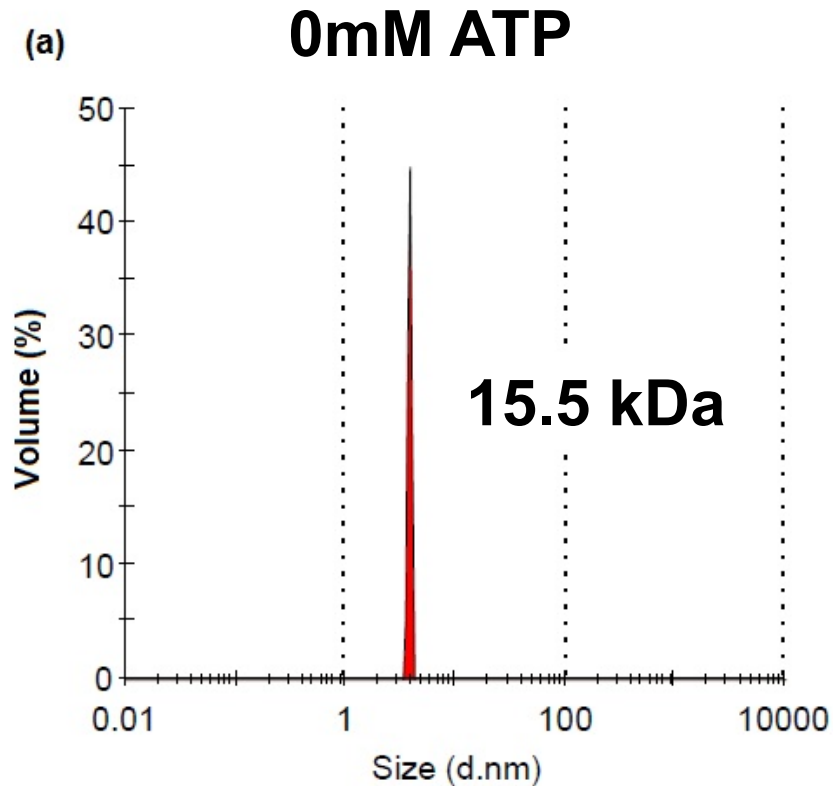
The changes in fluorescence emission of native Mb as a function of the ATP concentration



ATP-induced changes in intensity of maximum fluorescence emission at 330 nm of native Mb



Effect of ATP on the size distribution of native Mb



Measurement condition 0.1M KCl 20mM Tris-HCl (pH 7.5) 15°C
Mbs;1.65mg/ml

Analyzed by malvern zeta sizer nano (dynamic light-scattering).

The molecular size and zeta potential of tuna Mb with or without ATP

Zeta-potential measurements for Mb in 0.1M KCl,20mM Tris-HCl (pH7.5)

ATP concentration (mM)	molecular size (kDa)	Zeta potential (mV)
0	15.5	-4.50
5	11.3	1.32

Measurement condition 0.1M KCl 20mM Tris-HCl (pH 7.5) 15°C

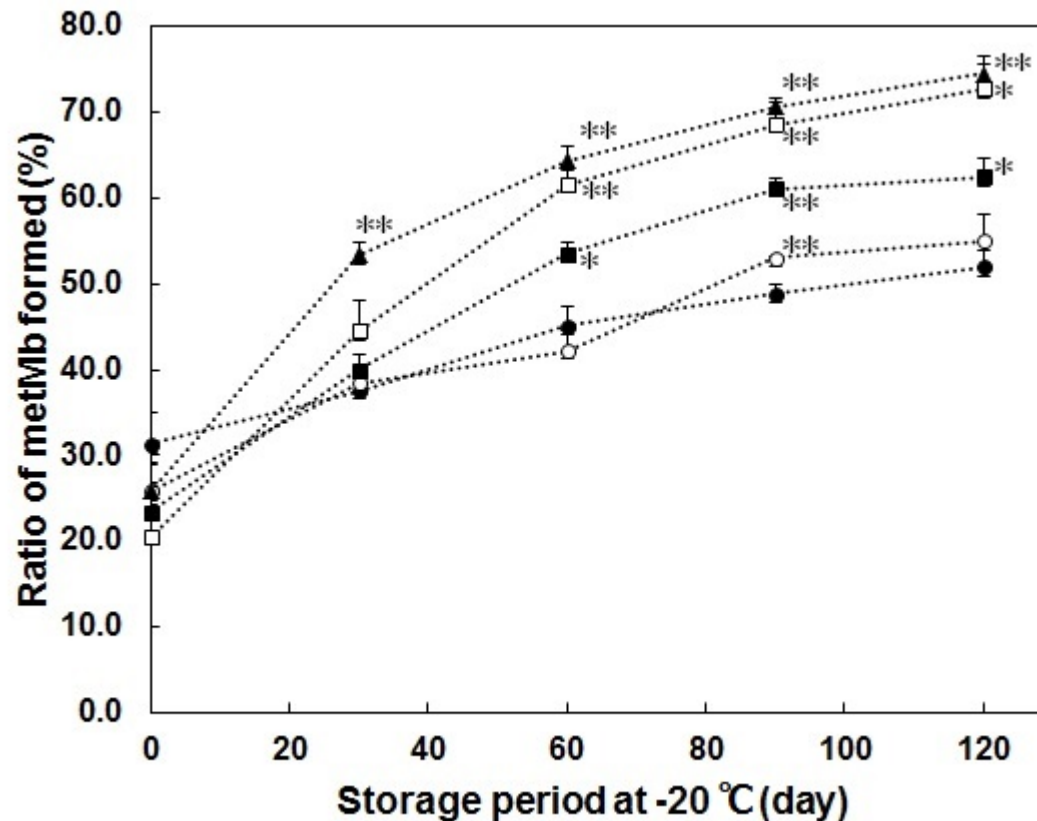
Mb;1.65mg/ml

Analyzed by malvern zeta sizer nano

Conclusions

- 1) ATP prevents autoxidation of Mb from oxyMb to metMb**
- 2) ATP induces the changes in molecular state of tuna Mb in solution.**
 - Visible absorbance spectrum**
The decreasing absorption of native Mb at solet region spectra.
 - Circular dichroism spectrum**
Increase in amplitude of maximum ellipticity in around 260nm with ATP.
 - Fluorescence spectrum**
The ATP-induced decrease in intensity of maximum fluorescence emission at 330nm.
 - Molecular size in solution and charge of Mb surface**
The molecular size and surface potential for tuna Mb changed by the presence of ATP.

ATP prevents discoloration of amberjack dark meat at -20 °C ?



Effect of postmortem time in iced water until freezing on metMb formation during frozen storage at -20°C for four months.

Postmortem time

(●) 1h, (○) 2h, (■) 3h,
(□) 5h, (▲) 7h.

**: (p < 0.01) Significant difference with respect to the 1h sample.

*: (p < 0.05) significant difference with respect to the 1h sample.

The progress of autoxidation of myoglobin was suppressed in the fillets containing high concentrations of ATP.

Development of stress-less harvest system from crawl and automatic high-speed fish treatment machine for cultured yellowtail

Electronic shock treatment



- ① stress-less harvest and instantaneous killing system (High conc. ATP, High quality meat, manpower saving)
- ② automatic high-speed fish treatment machine (high speed treatment, manpower saving, high efficiency, hygiene)
- ③ frozen & fresh fillet products with high concn. ATP

Stress-less harvest

High performance Killing machine

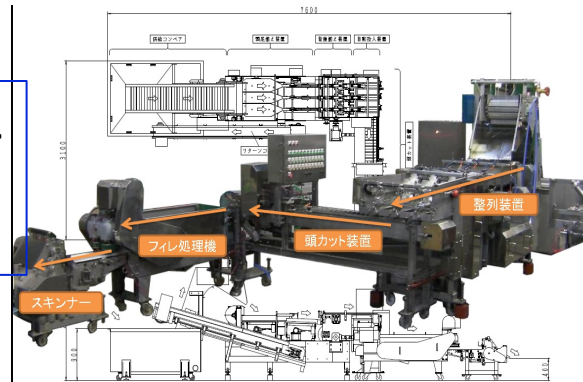


High conc.
ATP
Cooling

Make global distribution possible

- Without CO-treatment
 - High quality as sashimi
 - Distribution at -20~-25C
- produce high value at anywhere in the world

Automatic high-speed machine



Fillet contained high ATP



Quick frozen

Make distribution of
yellowtail fillet possible
at -20C



Frozen fillet contained ATP

Acknowledgement

This research was supported by grants from the Project of the NARO Bio-oriented Technology Research Advancement Institution (The Special Scheme to Create Dynamism in Agriculture, Forestry and Fisheries through Deploying Highly Advanced Technology).

Thank you for the opportunity to speak at the APEC conference in Kagoshima.