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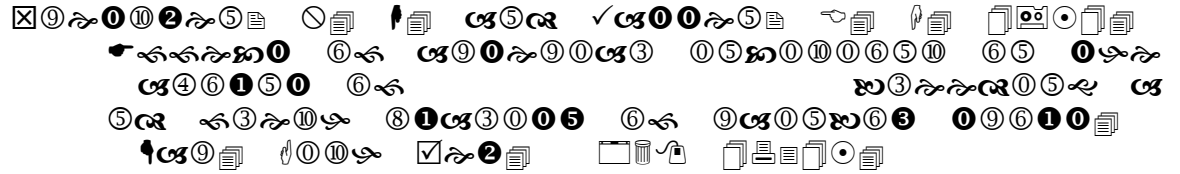

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APPENDIX

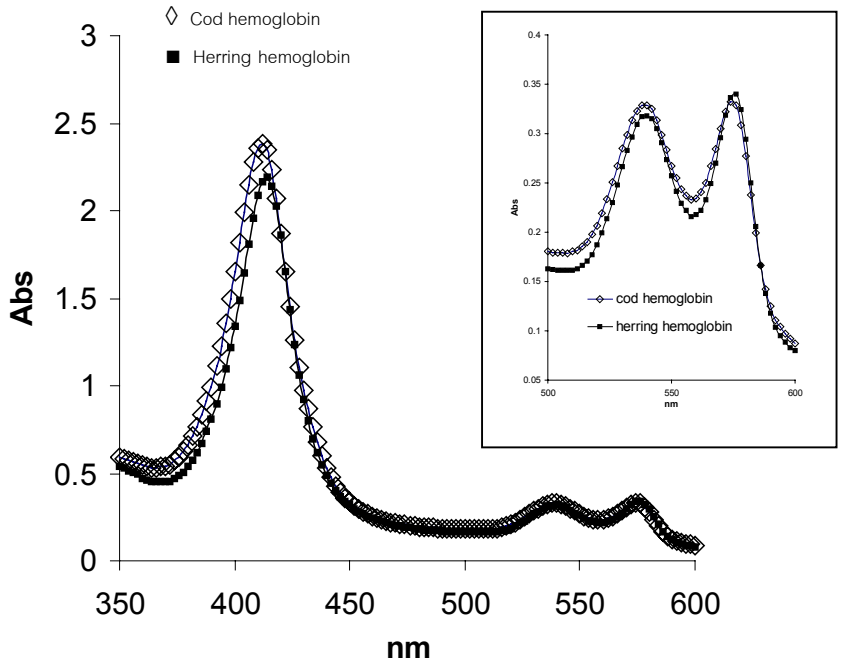

Figure 1 Typical visible light absorbance spectrum of cod and herring hemoglobins in 50 mM phosphate at pH 8.0.

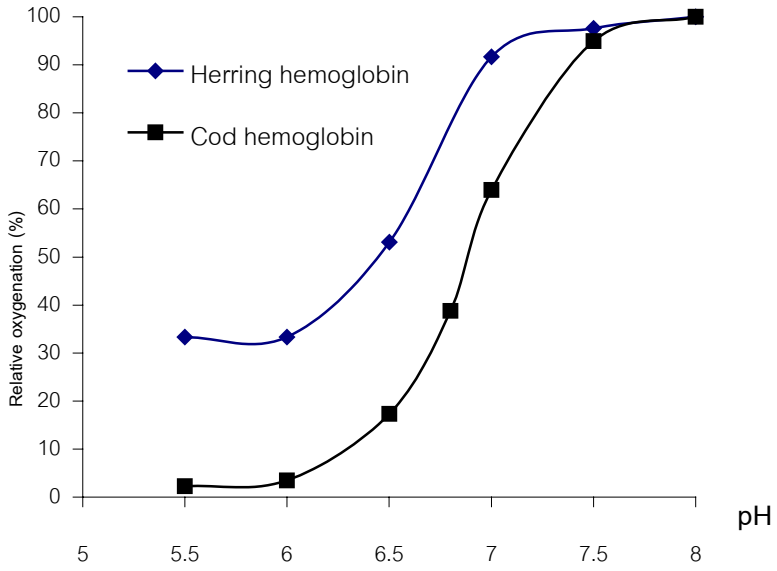

Figure 2 Relative oxygenation of cod and herring hemoglobin at various pH values.

Cod and herring hemoglobin (6.5 μM) in 50 mM sodium phosphate buffer at variuos pH values. The solutions were incubated in an ice bath for 10 min after dilution and scanned for their absorbance spectrum. Relative oxygenation was a product of 1,000 with a difference between a peak (~574nm) and a valley (~559nm) of the spectrum. A 100% of the relative oxygenation was the relative oxygenation of the solution at pH 8.0.

Table 1 Effect of the sarcoplasmic reticulum of cod or herring on the percentage of insoluble hemoglobin after incubation at pH 6.0 or 7.0 for 30 min.

Sarcoplasmic reticulum source	Per cent insoluble cod hemoglobin		Per cent insoluble herring hemoglobin	
	pH 6.0	pH 7.0	pH 6.0	pH 7.0
Cod	29.0 ± 4.7[1]	7.2 ± 2.0	6.7 ± 0.7	1.6 ± 0.6
Herring	12.3 ± 5.3	3.3 ± 0.9	22.1 ± 1.9	1.6 ± 1.4

The samples were 0.8 μM of cod or herring hemoglobin in 10 mM phosphate buffer (pH 6.0 or 7.0) with added sarcoplasmic reticulum (0.14 mg protein/ml) either from cod or herring. The mixtures were incubated in an ice bath for 30 min and then centrifuged at 186,000xg for 15 min. The soluble hemoglobin of the supernatants was quantified by measuring their absorbance values at 412 nm (A_{412}). The controls were the hemoglobin solutions without the added membrane but receiving identical treatment. The A_{412} of the cod and herring hemoglobin solution (without SR) at pH 7.0 before the treatments were 0.296 ± 0.003 and 0.268 ± 0.007, respectively, and after incubation at pH 6.0 and readjusting to pH 7.0 were 0.245 ± 0.004 and 0.257 ± 0.005, respectively. The insoluble hemoglobin caused by the treatment was based on the difference between A_{412} of the samples and those of the corresponding controls.

[1]Mean ± SD from duplicate experiments.

Table 2 Effect of hemoglobin addition at pH 10.8 on the per cent soluble hemoglobin remaining after the centrifugation at various precipitate pH values.

pH	Soluble hemoglobin (%)	
	Adding hemoglobin before the centrifugation at pH 10.8	Adding hemoglobin after the centrifugation at pH 10.8
7.0	62.1 ± 9.8[1]	83.6 ± 6.3
6.0	50.2 ± 10.6	67.4 ± 7.9
5.5	42.9 ± 8.2	54.3 ± 4.5

The cod homogenate without added hemoglobin was prepared. After adjusting pH of the homogenate to 10.8, hemoglobin (1 μM) was added either before or after the centrifugation at 104,630xg for 30 min. pH of these samples was readjusted to 7.0, 6.0 or 5.5 and centrifuged at the identical condition. The per cent soluble hemoglobin was calculated.
[1]Mean ± SD from triplicate experiments.

Table 3 Effect of existence of cod muscle soluble fraction (press juice) on per cent insoluble hemoglobin treated with the alkaline solubilization process.

Precipitate pH	Insoluble hemoglobin (%)

	10 % press juice	25 % press juice
7.0	3.9 ± 2.5[1]	31.8 ± 3.8
6.0	4.7 ± 3.6	32.8 ± 7.0
5.5	2.5 ± 3.5	37.8 ± 0.4

The samples were the cod hemoglobin solutions (1.0 μM in 20 mM $NaHCO_3$) with 10% or 25% the cod muscle soluble fraction or press juice. They were incubated in an ice bath at pH 10.8 for 30 min and adjusted to pH 7.0, 6.0 or 5.5 with 1 N HCl. The insoluble component was removed by centrifugation at 186,000xg for 15 min. Soluble hemoglobin in the samples after the treatments was quantified. Soluble hemoglobin of the solutions without added press juice of each treatment was 100% soluble hemoglobin. Heme protein content of the press juice was 1.4 ± 0.2 μM.

[1]Mean ± SD from duplicate experiments.

Table 4 Effect of freezing on extractable heme protein from light and whole muscle of herring

Sample	Extractable heme protein (μmole/kg)	
	Light muscle	Whole muscle
Fresh fish	11.1 ± 2.6	20.9 ± 3.2
Thawed fish	9.8 ± 1.6	21.1 ± 4.8

Fresh herring was frozen at -45^{0}C and stored at this temperature for 7 days. It was thawed overnight in chilled room (13^{0}C) before heme protein extraction. The extraction was performed and extractable heme protein was quantified.

[1]Mean ± SD of twelve fish.