Extraction and Analysis of Ommochromes in Cephalopod Chromatophores

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Introduction: Cephalopods are a type of mollusk that are known for their large heads, large eyes, and prehensile tentacles. Examples of cephalopods are squids, cuttlefish, and octopus. They possess the unique ability of changing their skin color to match their surroundings, and can thus camouflage with their environment. This color change is due to the contraction and relaxation of chromatophores, which are pigment sacs that contain proteins and ommochromes. Ommochromes are visual biological pigment molecules known for their contribution to the color of insects and crustaceans. The goal of this project is to confirm whether ommochromes are present in cephalopod chromatophores,

and to develop reliable methods for their extraction and elucidation.

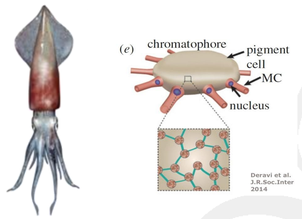


Figure 1: Lonfin Inshore Squid and illustration of chromatophore organ.

Long Term Goal: The long term goal is to apply the unique properties of ommochromes to advanced electronics and clothing. Examples would include roll able televisions and clothing that would camouflage the wearer to match their surroundings causing them to "vanish".

Experimental: Longfin inshore squid were decapitated and stripped of their chromatophore-containing skin layer. Excess flesh was broken down via washes with homogenization buffer and papain/collegenase, isolating the chromatophores. The pigment granules were washed with 5% w/v HCl-MeOH until no more color was extracted. SEM images were taken before and after the acidic methanol washes. The extract was separated on glass silica TLC plates with 3:1 phenol and water. The separated bands were "scraped" off the TLC plate and re-extracted with acidic methanol. The isolated band solutions were analyzed via UV/Vis and Mass Spectrometry.

Results:

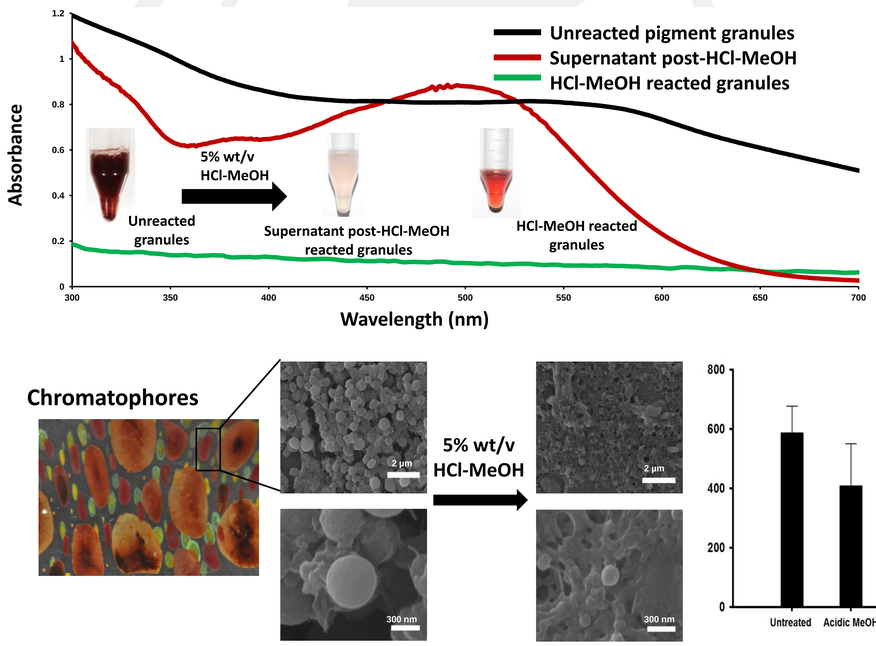
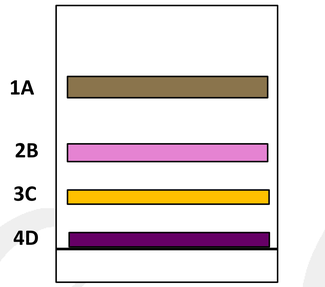
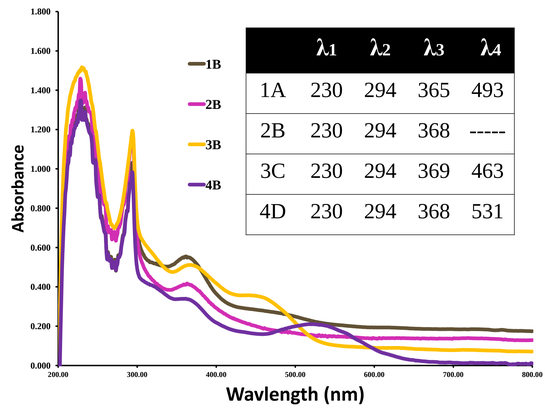


Figure 2: Absorption and SEM of pigment granules pre- and post-extraction



Band	Rf	σ
1A (brown)	0.56	0.01
2B (pink)	0.40	0.02
3C (orange)	0.33	0.01
4B (red-purple)	0.090	0.005
Xanthommatin (red-orange)	0.36	___
3-Hydroxy-kynurenine	0.56	___

Figure 3: TLC separation and the Rf values. Compared to Rf values from Nijhout.



	λ1	λ2	λ3	λ4
1A	230	294	365	493
2B	230	294	368	-----
3C	230	294	369	463
4D	230	294	368	531

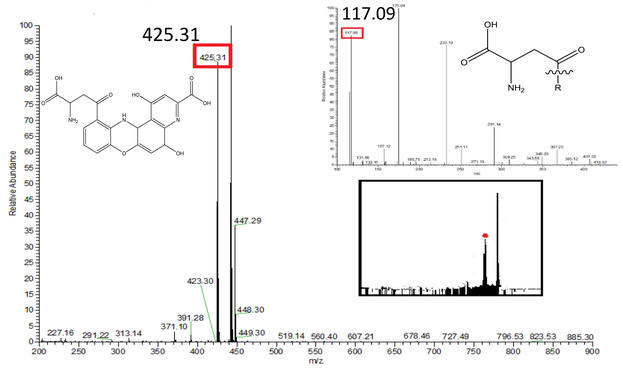


Figure 5: Mass Spectra of 2B. Peak 425.31 matches Xanthommatin.

Discussion: The SEM and absorption data showed that the extraction method pulled off the color compound in the pigment granules (figure 1), and that the coloration is mainly from the pigmented ommochromes. The TLC separation proved effective, giving similar Rf values to some ommochromes found in insects (figure 2). Each band in the absorbance data contained a unique shoulder and could help narrow down structures(figure 3). The mass spectra shows that xanthommatin is in 2B (pink) (figure 4). The other mass spectra contained "strange" peaks that will need further experimentation to identify.

Conclusion: Acidic methanol is effective at pulling off the ommochromes. The color of the chromatophores comes mainly from the pigmented ommochromes. Xanthommatin is present in cephalopod chromatophores.

Continued Research: A future plan is to try using reverse phase TLC to check for further separation of the ommochromes. With further separation, NMR can be used to identify the other ommochromes. Lastly, Redox chemistry can be used to help identify the chemical characteristics of the ommochromes.

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References:

1. Deravi LF, Magyar AP, Sheehy SP, Bell GR, Mäthger LM, Senft SL, Wardill TJ, Lane WS, Kuzirian AM, Hanlon RT, Hu EL,Parker KK. The structure–function relationships of a natural nanoscale photonic device in cuttlefish chromatophores. J. R. Soc. Interface 11: 2014.
2. Nijhout, H.F. Ommochrome Pigmentaion of the linea and rosa Seasonal Forms of Precis coenia (Lepidoptera: Nymphalidae). Archives of Insect Biochemistry and Physiology 36:215–222: 1997
3. Shamim G, Ranjan S, Pandey D, Ramani R. Biochemistry and biosynthesis of insect pigments. European Journal Of Entomology April 2014;111(2):149-164.