Elucidation of pigment-binding proteins, the development of the midwestern blot

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Abstract

Monarch butterflies (*Danaus plexippus*) are a distinctive and beloved species in part due to their unique wing coloration. Three novel deaminated xanthommatin variants were recently discovered among monarch wing pigments, and these variant compounds have not been identified in any other organism. The existence of these three compounds implies the existence of presently unknown enzyme(s) which catalyze the pigments' biosynthesis. The western blot and its modified technique the far western blot are useful tools for identifying proteins of interest and protein-protein interactions using antibodies and bait-proteins, respectively. The ultimate aim of this project is to identify the enzymes which catalyze the transformation of xanthommatin to deaminated xanthommatin derivatives. To achieve this goal, we have developed a new technique to aid in isolating and characterizing pigment-binding proteins, called the midwestern blot. The midwestern blot identifies interacting proteins that bind to a pigment ligand. We are currently validating the midwestern blot using the heme binding proteins myoglobin and cytochrome c. Following validation, the midwestern will be used to isolate xanthommatin-binding proteins extracted from monarch butterfly pupae. The isolated proteins will then be characterized using mass spectrometry and chromatography techniques. Our results indicate that the midwestern blot is a promising pigment-binding protein identification technique, with signal-to-noise visible to the naked eye with both myoglobin and potentially other heme binding proteins. In addition to providing a new tool for the observation of pigment-binding proteins from a mixture, the results of this work will increase the current understanding of the endangered monarch butterfly and may provide insight as to the evolution and mechanism of this deamination synthesis.

Introduction

- Endangered monarch butterflies (*Danaus plexippus*) use their distinctive wing coloration to ward off predators and for sexual selection[1].
- **Novel pigment compounds** were identified in monarch butterfly wings, including three deaminated variants of known wing pigment xanthommatin[2]
- These deaminated xanthommatins absorb light differently in UV and the visible spectrum.
- **The existence of the three novel xanthommatins implies the existence of presently unknown enzyme(s) which catalyze the synthesis of these pigments.**
- Current protein blotting techniques (e.g., Western, Far-Western) use radio- or fluorescent-labeled antibodies or proteins to isolate proteins of interest.

Could the pigment xanthommatin itself can help us visually identify what proteins it binds to, without the need for radio- or fluorescent-labeling techniques?

Research Questions and Aims

Question: What enzyme(s) catalyze the transformation of xanthommatin to deaminated xanthommatin?
Problem: How do we find proteins that bind to the known pigment ligand xanthommatin?

Aim 1: Develop and validate the midwestern blot

Aim 2: Use midwestern to isolate xanthommatin-binding proteins from monarch pupae

Aim 3: Identify xanthommatin-binding proteins

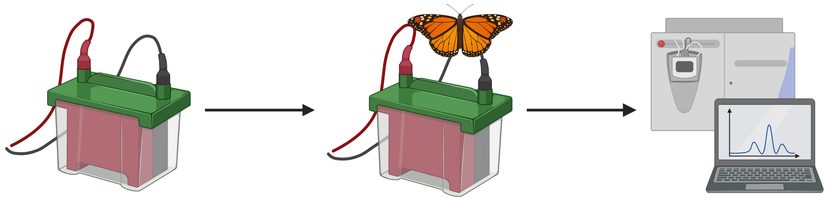

Figure 1. Aims of this project, ultimate goal of which is identifying xanthommatin-binding proteins from monarch butterfly pupae. The current poster focuses on aim 1.

Methods

Aim 1: Develop and validate the midwestern blot using heme-binding proteins myoglobin, hemoglobin, and catalase as known test proteins

1. Separate proteins from their heme ligand, according to Teale et al.
2. Separate proteins using denaturing SDS-PAGE
3. Transfer to PVDF membrane
4. Block membrane using 2% BSA
5. Incubate overnight in 1 mg/mL solution of hemin in DMSO with BSA

Midwestern blot workflow

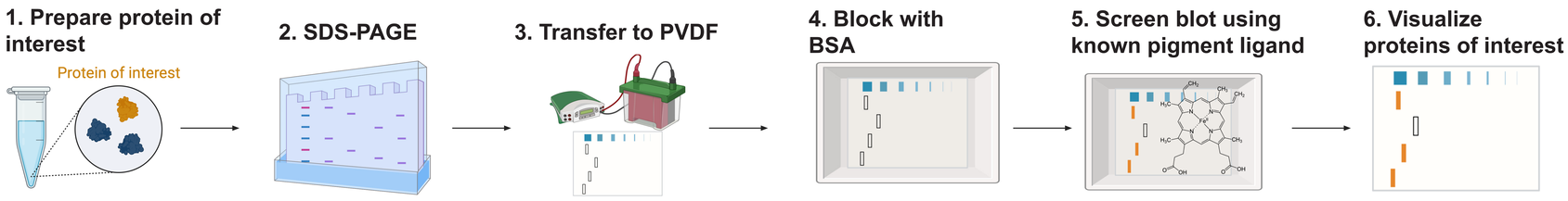

Figure 2. Protocol for conducting a midwestern blot on any pigment binding protein.

Data

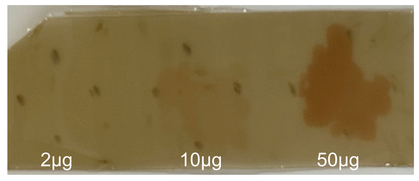

Figure 3. Dot blot of apomyoglobin for midwestern proof of concept. Apomyoglobin was spotted onto wet PVDF, then blocked for 1 hour with 2% BSA. The membrane was then incubated in 1mg/mL hemin solution overnight.

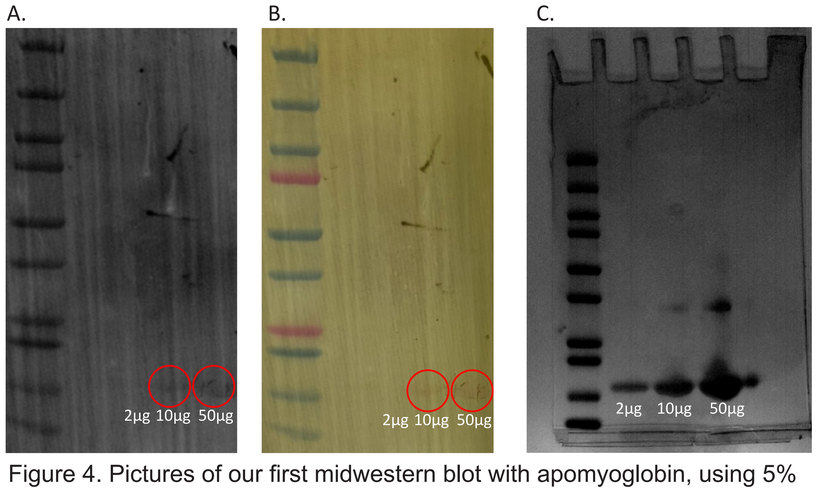

Figure 4. Pictures of our first midwestern blot with apomyoglobin, using 5% milk as a blocking agent.
Panels A and B are images of the same blot; A was taken with a gel imager and B was taken using an iPhone camera. The signal-to-noise is visible on the 10µg and 50µg bands with the naked eye.
Panel C shows the SDS-PAGE gel with all three bands (2µg, 10µg, and 50µg) visible with staining.

Discussion

- Our first experiments had a significant background noise problem when using milk as a blocking agent (figure 4)
- After conducting a series of dot blots testing the efficacy of different combinations of blocking agents, we determined that the most effective blocking method for midwestern blots using hemin pigments is 2% BSA in PBS, both before incubation with the pigment, and during incubation
- Interestingly, we have noted that the signal-to-noise ratio increases as the blot dries
- The current project expands upon existing blotting techniques and may shed light on the biological synthesis of xanthommatin derivatives in monarch butterflies

Future work

- Due to covalent interactions we have been unable to separate cytochrome C from its heme. Thus, we have decided to use catalase as our third test protein instead of cytochrome C
- Validate the midwestern using native electrophoresis in addition to SDS-PAGE
- Once the midwestern blot has been validated, we will apply the midwestern blot to pigments whose proteins are not known, including xanthommatin and its novel derivatives

Acknowledgements and references

T.C.B. was supported by UVU Honors, UVU College of Science Scholarly Activities Committee, and the UVU Chemistry Department during this project.

References and contact information can be found on the author's website: tessacblack.github.io