Characterization of proteins involved in biotransformation

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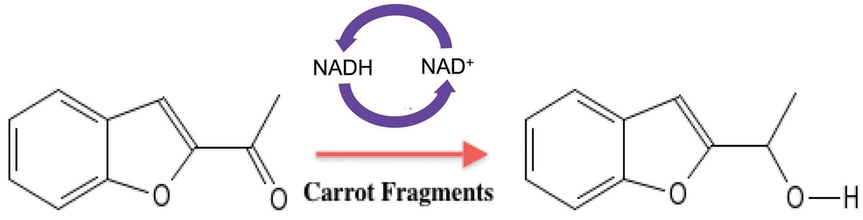
ABSTRACT

The biotransformation reaction of benzofuran-2-yl methylketone to 1S-1(2-benzofuranyl)-ethanol has been investigated and characterized; however, the specific enzyme responsible for the conversion has not been previously isolated. In an attempt to isolate the enzyme from the surface of the carrot strips, the strips were incubated in various buffers and detergents under gentle or more vigorous mixing. The resulting protein solutions were then concentrated and further analyzed using Bradford assay and SDS-PAGE. Bradford analysis of all samples indicated a protein concentration range of 375-455 µg/ml, and at least four protein bands were present in SDS-PAGE.

REACTION



PROTEIN ANALYSIS

METHODS:

- Carrot strips were mixed with various buffers on either a rotisserie or vortexer.
- These samples were then filtered and the filtrate collected.
- The filtrate was concentrated 20 to 25 fold using Amicon's 3kD MWCO Centrifugal Concentrators and further analyzed using SDS-PAGE and Bradford assay.

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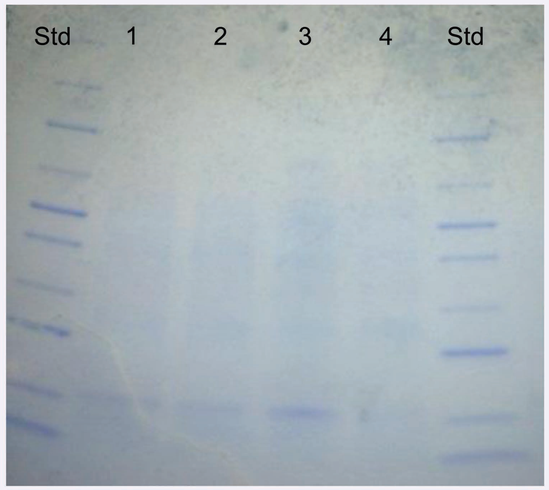
Table 1: Solutions of carrot samples prepared for Bradford and SDS-PAGE analysis.

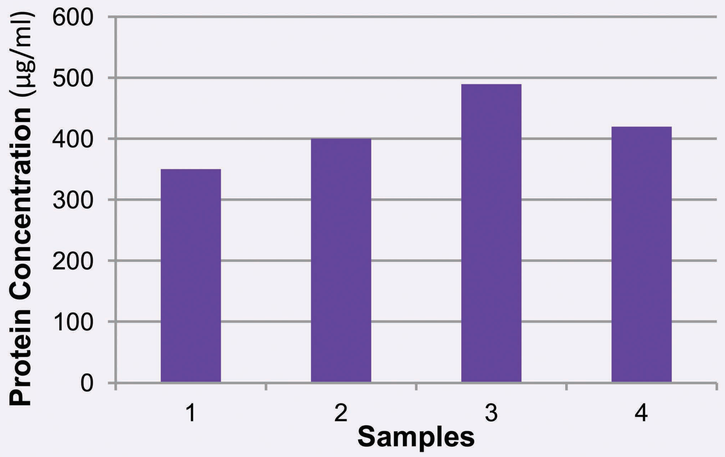
Sample	Buffer Used	Method of Agitation
1	Carbonate at pH 10	Rotisserie
2	1X TBS at pH 7	
3	Carbonate at pH 10	Vortex
4	1X TBS at pH 7	

Figure 1: SDS-PAGE results for the **concentrated** filtrates of the prepared samples.



The SDS-PAGE confirmed the presence of proteins, although the specific bands are hard to distinguish.

Figure 2: Bradford assay results for the **concentrated** filtrates of the Samples 1-4 (as shown in Table 1.)



ADDITIONAL STUDIES

Study 1: How much carrot is necessary for the conversion of ketone to alcohol?
Method: 16 samples were prepared, each differing by 0.5 g with 25 ml of dH_2O and 25 mg of ketone. Presence of alcohol confirmed with TLC.
Conclusion: Only 4 g of carrot is necessary for complete conversion of 25 mg ketone to alcohol.

Study 2: How many conversions are possible with a single 8 g sample of carrot strips?
Method: 8 g sample of carrots with 25 ml dH_2O and 25 mg of ketone was prepared. 1 ml aliquot tested for the presence of alcohol using TLC. Used carrot strips were washed and placed in new sample tube with additional 25 ml $dH_2O$and 25 mg of ketone. (Process repeated 4 times.)
Conclusion: Only two complete conversions were possible with repeated use of a single carrot sample.

CONCLUSION

According to the results of SDS-PAGE and Bradford analysis, the carbonate buffer with vortexer resulted in the highest concentration of protein removed from the carrot. Although the protein was not isolated, the additional studies were carried out to gain a better understanding of the properties of the protein and its dependence on the cofactor. There appears to be enough cofactor in an 8 g sample of carrot strips for two complete conversions. Future plans include using cellulase to "loosen" surface of the carrot strips to attempt to remove more protein from the carrot surface for analysis.

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