I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM • XV SHEB • XV ENZITEC FLORIANÓPOLIS • 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
Choose an item.

PURIFICATION AND PARTIAL CHARACTERIZATION OF PROTEINS EXTRACTED FROM PHOTOSYNTHETIC MICROORGANISMS

Thalya N. S. Santos[1], Maria Carla S. Arruda[1], Sara C. Silva[1], Yanara A. S. Moura[1], Pablo E. C. e Silva[1], Romero M. B. P. Costa[2], Daniela A. V. Marques[2], Luiza R. A. Lima[2], Ana L. F. Porto[1] & Raquel P. Bezerra[1]*

[1]*Department of Morphology and Animal Physiology, Rural Federal University of Pernambuco (UFRPE), Recife, Brazil.*

[2]*Institute of Biological Sciences, University of Pernambuco, Recife, Brazil*

* *Corresponding author's email address: raquel.rpbezerra@ufrpe.br*

ABSTRACT

Algae have a rich biochemical diversity producing bioactive compounds with a wide range of activities. Thus, purification techniques are crucial for reliable characterization. Therefore, this work is dedicated to purifying and partially characterizing a lectin extracted from the microalgae *Dunaliella tertiolecta* in order to identify its biochemical profile. The lectin was purified on a single chromatographic column (DEAE-Sephadex) and characterized regarding the influence of different temperatures at times of 30 and 60 minutes and divalent ions. Thus, the study indicated the efficiency of protein isolation, obtaining a purification factor of 291.04x and a yield of 15% with relative hemagglutinating activities >4,096 UH/mL. The temperature test revealed the instability of the lectin in the face of greater heat ranges, with complete denaturation at 90 and 100ºC. Regarding ionic influence, the lectin in question does not have ion-dependent activity, however, it can be potentiated by $ZnCl_2$, FeC_{l3}, $CuSO_4$ and $NaSO_4$ and inhibited in the presence of K_2SO_4, $CaCl_2$ and $MgSO_4$. In conclusion, the initial results obtained allow us to infer optimistic predictions regarding the biochemical profile of the lectin, but new tests and studies need to be carried out to better direct its application in the future.

Keywords: Lectins. Characterization. Microalgae.

1 INTRODUCTION

Algae are a widely diverse group of organisms, including numerous marine and freshwater species [1]. Have extremely important ecological roles, since they are primary producers, produce oxygen and contribute strongly to the maintenance of the ecosystem they inhabit [2]. In addition, compared to terrestrial plants, algae have high growth rates, photosynthetic efficiency and rich biochemical diversity. Such characteristics highlight algae as a potential raw material for the production of biomolecules of industrial, nutraceutical and pharmaceutical interest [3, 4]. Bioactive compounds derived from algae included lipids, vitamins, proteins [5][6]. Among the proteins produced are lectin, lectins are a group with proteins with a non-immune origin that displaying a high specific affinity for carbohydrates [7] lectins can be purified from different sources, including plants, animals, fungi and algae, that can be applied to different fields biochemicals and biologicals [8]. Natural therapeutic compounds such as nucleic acids, proteins, lipids and carbohydrates have already been isolated from algae with a wide variety of biological functions: antitumor, anti-inflammatory, antioxidant, antifungal and others [9, 10]. In this sense, obtaining and correctly isolating proteins using precipitation, chromatography and electrophoresis techniques is of utmost importance for their study and potential applications [11].

2 MATERIAL & METHODS

2.1 Preparation of extract

Lyophilized biomass (1g) was homogenized in 10 mL of 0.01M PBS pH7.15 buffer added with 1 mL of Tween 80 for an interval of 2-4h at room temperature, centrifuged (4500 rpm/10 min) and the supernatant was used for hemagglutinating activity and the following steps.

2.2 Lectin purification

Supernatant (crude extract) of *D. tertiolecta* was precipitated with cold absolute ethanol (1:3) for 10 minutes, centrifuged (4500 rpm/20 min), resuspended in 0.15 M NaCl and loaded into anion exchange chromatography using DEAE-Sephadex. Elution was carried out at a flow rate of 1 mL min^{-1} in Tris-HCl-NaCL buffer pH 7.5 0.1M. The collected fractions were monitored by spectrophotometry with an absorbance of 280 nm and the hemagglutinating activity was measured.

2.3 Total protein quantification

Protein content of the samples was determined by using BCA protein assay reagent Kit (BCA™ Protein Assay Kit, Thermo SCIENTIFIC). Bovine serum albumin was used as standard protein. Quantification is carried out using the ELISA reader at a wavelength of 595 nm (Smith et al., 1985).

2.4 Determination of Hemagglutinating Activity (HA)

The hemagglutinating activity was performed in microtiter plates according to Correia & Coelho (1995). This test allows you to visually indicate the presence of lectins capable of agglutinating erythrocytes. Rabbit and human type O and A red blood cells treated with glutaraldehyde were used to perform the test.

2.5 Effect of temperature on lectin stability

A series of thermal treatments were carried out at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C and 100 °C for 30 min and 60 min. Subsequently, hemagglutinating activity was performed to verify the stability of the lectin during the assay.

2.6 Effect of íons on activity on lectin

Lectin serial dilution was carried out in 100 Mm solutions of the respective ions: K_2SO_4, $CaCl_2$, $MgSO_4$, $KHSO_3$, $ZnCl_2$, $FeCl_3$, Na_2SO_4, $CuSO_4$ in a proportion (1:1). Hemagglutinating Activity was used to analyze lectin activity. Finally, the lectin without the presence of íons was adopted as a positive control.

3 RESULTS & DISCUSSION

The lectin from *Dunaliella tertiolecta* was purified using a combination of ethanol precipitation and the DEAE-Sephadex ion exchange column. Figure 1 shows the chromatogram, which exhibited one single peak (fractions 27 - 34). These protein fractions were pooled and concentrated showing relative hemagglutinate activity >4,096 mg/mL and specific activity >258,700 UH/mL. After purification, the lectin was 261-fold purified with a yield of 15% (Table 1).

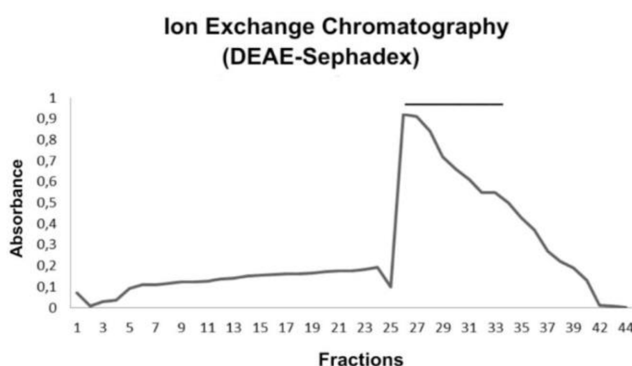

Figure.1: Purification of the hemagglutinating protein from the microalgae *D. tertiolecta* by anion exchange chromatography on a DEAE-Sephadex column. (-) Fraction collected.

Sample	Volume (mL)	Total Proteins (mg)	hemagglutinating activity (UH/mL)	Specific Activity (mg/mL)	Yield (%)	Purification factor
Extract	11	1,584	1,408	>888.89	100	1
Precipitate	1.5	0.344	128	2.048	21.71	2.304
DEAE-Sephadex	15	0.238	>4,096	>258.700	15.00	291.04

Table.1: Purification steps of the hemagglutinating protein from the microalgae *D. tertiolecta*.

The lectin hemagglutinating activity was high at 20 ºC for 60 min, but was reduced (around 75%) at 30 ºC. When incubated at 40 ºC, the activity was slightly decreased and remained constant until 70 ºC. The lectin was completely denatured at 80 ºC. These results suggest an unstable behavior of the lectin, which can indicate a possible high molecular weight of the protein, since in general low molecular weight lectins are more thermostable due to their monomeric structure **[14]**. For example, the lectin (15 kDa) from *Chlorella pyrenoidosa* exhibited high stability, resisting at 90 ºC for 10 min **[15]**.

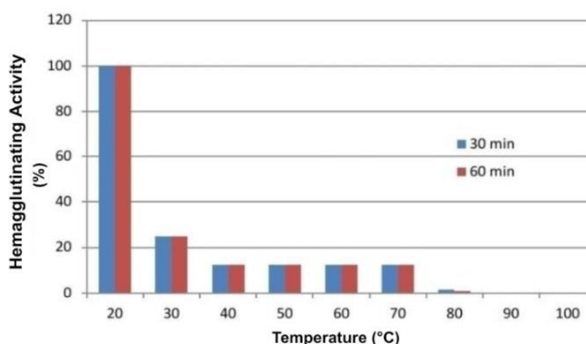

Figure 2: Relationship between temperatures and relative hemagglutinating activity of the lectin.

Moreover, the hamagglutinating activity was assessed for both rabbit and human blood (Table 2). The data showed that the lectin tested in rabbit blood was resistant to all ions tested, specially for $ZnCl_2$, $FeCl_3$, $CuSO_4$ and $NaSO_4$, showing high relative hemagglutinating activity (> 4,096). In contrast, when tested for human blood, the hemagglutinating activity was inhibited by the ions K_2SO_4, $CaCl_2$ and $MgSO_4$, when compared to the initial hemagglutinating activity (256 U). These results indicate that the lectin from *D. tertiolecta* showed a higher affinity with rabbit erythrocytes (v/v) since they have a low amount of siliatic acids on their surface. The same characteristic occurs in type O human blood, however, in other blood groups (A, B and AB) the low amount of siliatic acids is not observed **[17]**. Finally, although the hemagglutinating activity is enhanced or inhibited in the presence of ions, the lectin from *D. tertiolecta* does not have ionic dependence, since it does not need these agents to bind to glycoproteins present on the surface of red blood cells. This characteristic also occurs in the lectins obtained from *Hypnea musciformis* **[18]** and *Bryothamnion triquetrum* **[19]** macroalgae.

Íons	Activity in human blood (A) (UH/mL)	Activity in rabbit blood (UH/mL)	Control (UH/mL)
K_2SO_4	2	256	
$CaCl_2$	0	512	
$MgSO_4$	8	256	
$KHSO_3$	128	512	
$ZnCl_2$	>4,096	>4,096	
$FeCl_3$	>4,096	>4,096	
Na_2SO_4	256	>4,096	
$CuSO_4$	>4,096	>4,096	256

Table.2: Hemagglutinating activity in the presence of ions (100mM).

4 CONCLUSION

The lectin from microalgae *Dunaliella tertiolecta* exhibited high purity and high hemagglutinating activities. From this study, it was also verified that the lectin is not thermostable, which allows us to infer its probable high molecular weight, since an electrophoretic test is necessary to confirm this. In addition, the results obtained demonstrate that the lectin in question does not have ionic dependence, but can be potentiated or inhibited in the presence of these. Finally, to better understand the biotechnological potential of *Dunaliella t.* The other tests for its full characterization must be carried out.

REFERENCES

1. DINABANDHU, S., SECKBACH, J. 2015. The algae world. 26 (11564). 3-307
2. POONAM, S., NIVEDITA, S. 2017. Industrial and biotechnological applications of algae: a review. 1 (1). 01-25
3. STEPHENSON, P. G., MOORE, C. M., TERRY, M. ZUBKOV, M. V., BIBBY, T. S. 2011. Trends in biotechnology, 29 (12), 615-623
4. WU, J. Y., TSO, R., TEO, H. S., & HALDAR, S. 2023. Frontiers in Nutrition, 10.
5. CHEN, F., LENG, Y., LU, Q. e ZHOU, W. 2021. Algal Res 60: 102541.
6. CHEN, H., WANG, Q. 2022. Frontiers in Microbiology. 13, 832097.
7. MALIKli, I. M., MISSON, M., Teoh, P. L., RODRIGUES, K. F., & YONG, W. T. L. 2022. Marine Drugs. 20 (2). 102.
8. PEUMANS, W. J., & VAN DAMME, E. J. 1995. Plant physiology, 109 (2), 347.
9. CARVALHO, L. G., & PEREIRA, L. (2014). Marine Algae, 195-227.
10. SHALABY, E. (2011). *Plant signaling & behavior*. 6 (9). 1338-1350.
11. SINGH, R. S., & WALIA, A. K. (2018)l. Journal of applied phycology. 30,1833-1858
12. SINGH, R. S., WALIA, A. K., KHATTAR, J. S., SINGH, D. P., KENNEDY, J. F. 2017. International Journal of Biological Macromolecules. 102, 475-496.
13. SHETTY, V. SIBI, G. 2015. Food Resource sci. 4. 1-9.
14. NAGANO, C. S., MORENO, F. B. M. B., BLOCH Jr, C., PRATES, M. V., CALVETE, J. J., SAKER-SAMPAIO, S., SAMPAIO, A. H. (2002). Protein and Peptide Letters. 9 (2), 159-165.
15. ZHENG Yi, YU Ping, LIU Yan-ru. ACTA HYDROBIOLOGICA SINICA. 2003. 27(1):36-40.
16. SHARON, N. LIS, H. 2001. Molecular immunology of complex carbohydrates. 2.1-16.
17. BULAI, T. BRATOSIN, D. PONS, A. MONTREUIL, J. ZANETTA, JP. 2003. Biochemistry 42:2578-2584.
18. NAGANO, CS. MORENO, FBMB. BLOCH, C JR. PRATES, MV. CALVETE, JJ. SAKER-SAMPAIO, S. FARIAS, WRL. TAVARES, TD. NASCIMENTO, KS. GRANGEIRO, TB. CAVADA, BS. SAMPAIO, AH. 2002. Prot Pept Lett. 9: 159-165.
19. CALVETE, JJ. COSTA, FHF. SHAKER-SAMPAIO, S. MURCIANO, MPM. NAGANO, CS. CAVADA, BS. GRANGEIRO, TB. RAMOS, MV. Jr, CB. SILVEIRA, SB. FREITAS, BT. SAMPAIO, AH. 2000. Cell Mol Life Sci. 57:343-350.

ACKNOWLEDGEMENTS

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – [Finance Code 001] and by the Foundation for Science and Technology of the State of Pernambuco [FACEPE, APQ-0486-9.26 22; APQ-1480-2.08/22].