Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.5, No.20, 2015

Purification of G-Protein Coupled Receptor from Whole Cell of Local Strain of *Saccharomyces cerevisiae*

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Abstract
The aim of study To purify GPCR from a local strain of *S. cerevisiae* using Ion exchange and gel filtration chromatography techniques , by packing materials for columns which will be chosen of low cost comparing to the already used in published researches, which depend on the costly affinity chromatography and other expensive methods of purification. Local strain of *S. cerevisiae* chosen for extraction and purification of G-protein coupled receptor (GPCR) .The strains were obtained from biology department in Al- Mosul University, Iraq. The isolated colony was activated on Yeast Extract Pepton Dextrose Broth (YEPDB) and incubated at 30 C° for 24 h .Loop fully of the yeast culture was transferred to (10ml) of yeast extract peptone glucose agar (YEPGA) slant , then incubated at 30C°for 24h , after that it was stored at 4C° ,the yeast cultures were reactivated and persevered after each two weeks period. *S.cerevisiae* was identified by morphological, microscopic characterization and biochemical test . The GPCR that extract from whole cell of *S.cerevisiae* was purified by ion exchange chromatography using DEAE-Sepharose ,the bound proteins (negatively charged) were then eluted using gradient concentration of NaCl ranged between(0.1 -0.5M). Gel filtration chromatography using Sepharose 6B was applied as a second step of purification. The optical density for each fraction was measured at 280 nm by UV-VS spectrophotometer then the GPCR concentration was determined by using ELISA Kit . The fractions which gave the highest absorbance and concentration of GPCR were collected .The molecular weight of GPCR was determined by gel filtration chromatography using blue dextrin solution. Standard curve was plotted between log of molecular weight for standard protein and the ratio of Ve/Vo of GPCR . The purity of the GPCR that extracted and purified from whole cell of *S, cerevisiae* were carried out by using SDS-PAGE electrophoresis . In ion exchange chromatography the fraction were collected with 5 ml tube at a flow rate 0.5 ml/ min and eluted with gradient (0.1-0.5M) of sodium chloride solution. Two proteins peaks appeared after eluted by the gradient concentration of sodium chloride, while no protein peaks appeared in the washing fractions. The GPCR concentration was measured in the fractions of these two protein peaks, data indicated that GPCR located in the first protein peak (eluted at 0.1M of NaCl) at fraction numbers between 3 and 9, the maximum concentration of GPCR was 9.281 with specific activity 71.58(ng/mg)protein , 3.125 purification folds and72.9(%) yield while the second peaks (eluted at 0.4 M of NaCl) don't give any concentration for GPCR, thus its neglected. Gel filtration chromatography was used as second step of purification which applied by using sepharose 6B. Results show single active protein peaks appeared that identical with the peak of GPCR at fractions numbers(29-35). The maximum concentration of GPCR was 9.082 (ng/ml)was observed in these fractions. The specific activity for these fractions was 151.37 (ng/mg) protein with 6.608 purification folds and 39.64 (%) yield. The present study a chive a relatively high purification of GPCR from whole cell of a local strain *S. cerevisiae* with fold purification 6.608 and a yield of 39.64 % and molecular weight about~33KD.
Key words: GPCR purification, *S.Cerevisea*, whole cell .

1. Introduction:

G-protein coupled receptor (GPCR) are the largest family of membrane bound proteins that have seven membrane spanning domains connected by intracellular and extracellular domains. Due to their polypeptide chain passes seven times through the plasma membrane ,the GPCR are also called seven - trans membrane (7 T) receptors that present in all eukaryotic organisms (Salon 2011, Szczepek 2014).

A thousand human genes code for GPCR and they are involved in sensing a wide range of extracellular stimulation such as adrenergic receptors, dopamine receptors histamine receptors the light receptor rhodopsin and many odor and taste receptors(Cho 2012).

Recently , the ability of different ligands of on individual receptor to promote distinct patterns of cruller response- has gained important therapeutically beneficial signaling path ways over those leading to harmful effects (Wootten 2013).

The GPCR are divided into five families depending on their sequence and structural similarities: rhodopsin (familyA), secretin (family B), glutamate (familyC), adhesion and frizzled /Taste(Ding2013)

G protein -GDP complex binds closely to GPCR which the change in the conformation of the GPCR activates the G protein and GTP physically replaces the GDP bound to the alpha subunit resulting in dissociation of G protein subunits into two parts , GTP-bound alpha subunit and a beta -gamma dimer , which both have an independent capacity to signal forward through the activation or inhibition of effectors (Kolesnikov 2011).

Hydrolysis of GTP to GDP leads to signal termination and reassociation of the heterotimer : regulators of G -protein -signaling proteins enhance the intrinsic GTP as activity of the G_α subunit which is used as a activators for many hormones and enzymes(Katritch 2013).
Saccharomyces cerevisiae (S.Cerevisiae) ,which is type of yeast, is the best source for extraction and purification of GPCR due to its simplicity with respect to genetic manipulation , economical propagation , easy maintenance of stability(Junge 2008, Krishnan 2012).

Three GPCRs are known in S. cerevisiae Ste2,Ste3 and Gpr1 .Although Ste2 and Ste3 are both coupled to Gpr1 and activate the mating pathway, the sequence similarity between them is limited (Bonini *et al* 2013) .

At the level of the receptor Set2 and Set 3 are down regulated by hyperphosphorylation of several C-terminal residues , followed by ubiquitylation , internalization and degradation . At the level of the $G\alpha$ protein ,GPa1, desensitization depends on the GTPase stimulating protein which is a member of the RGS -protein family (Baltoumas *et al*2013)

Purification is a major challenge in the field of GPCR research because many GPCRs are denatured by detergents .Due to the difficulty of purifying GPCRs, Only a small number of them have been successfully reconstituted to date(Chac *et al* 2010)

The aim of the present study to purify GPCR from a local strain of *S. cerevisiae* using Ion exchange and gel filtration chromatography techniques , by packing materials for columns which will be chosen of low cost comparing to the already used in published researches, which depend on the costly affinity chromatography and other expensive methods of purification .

2.Materials and Methods:

Local strain of *S. cerevisiae* chosen for extraction and purification of G-protein coupled receptor (GPCR) .The strains were obtained from biology department in Al- Mosul university ,Iraq.

The isolated colony was activated on Yeast Extract peptone Glucose Broth (YEPD) and incubated at 30 C° for 24 h .Loop fully of the yeast culture was transferred to (10ml) of yeast extract peptone glucose agar (YEPDA) slant , then incubated at 30C°for 24h , after that it was stored at 4C° ,the yeast cultures were reactivated and persevered after each two weeks period(Barnett *et al* 1990)

S.cerevisiae was identified by morphological, microscopic characterization and biochemical test, the pellet cells were thawed and resuspended in extraction buffer (50 mM PBS buffer , PH = 8.0 , 100mM NaCl , 5mM $MgCl_2$, 1mM AEBSF) and added protease inhibitor cocktail (us 10 µl for 1ml) with gentle swirling on ice (Shi *et al* 2005).

The *S.cerevisiae* cells can disruption by mechanical methods (ultrasoniction) which three time (1 min operation, 1 min shutting) (duty cycle 80% , power 140 w) under cool conditions(D.K. Apar& B. Ozbek 2008) .

n-Dodecyl-β-D-maltoside (DDM) (1%) and 5mM β-mercaptoethanol were added to the lysed sample and stirred on ice for 1 h. Lysed sample was centrifuged for 1 h at 10000 rpm to remove insoluble cellular material(Shi *et al* 2005).

The GPCR that extract from whole cell of *S.cerevisiae* was purified by Ion exchange chromatography using DEAE-Sepharose ,the bound proteins (negatively charged) were then eluted using gradient concentration of NaCl ranged between(0.1 -0.5M).

Gel filtration chromatography using Sepharose 6B was applied as a second step of purification. Crude GPCR was eluted with PBS(PH=7.4)with DDM buffer . Aliquot of 5ml fraction were collected in each tube with flow rate of 30 ml / h . The concentrated sample was applied to the sepharose 6B column that was pre equilibrated with (50 mM PBS PH = 7.4).Aliquot of an eluted with PBS(PH=7.4)with DDM buffer .5ml fractions were collected in each tube with flow rate of 30 ml/h . The optical density for each fraction was measured at 280 nm by UV-VIS spectrophotometer then the GPCR concentration was determined by using ELISA Kit from BlueGene Biotech, Shanghai, China. The fractions which gave the highest absorbance and concentration of GPCR were collected.

The molecular weight of GPCR was determined by gel filtration chromatography using blue dextrin solution .Standard curve was plotted between log of molecular weight for standard protein and the ratio of Ve/Vo of GPCR(StellWagen 1990).The purity of the GPCR that extracted and purified from whole cell of *S, cerevisiae* were carried out by using SDS-PAGE electrophoresis(Garfin 2003).

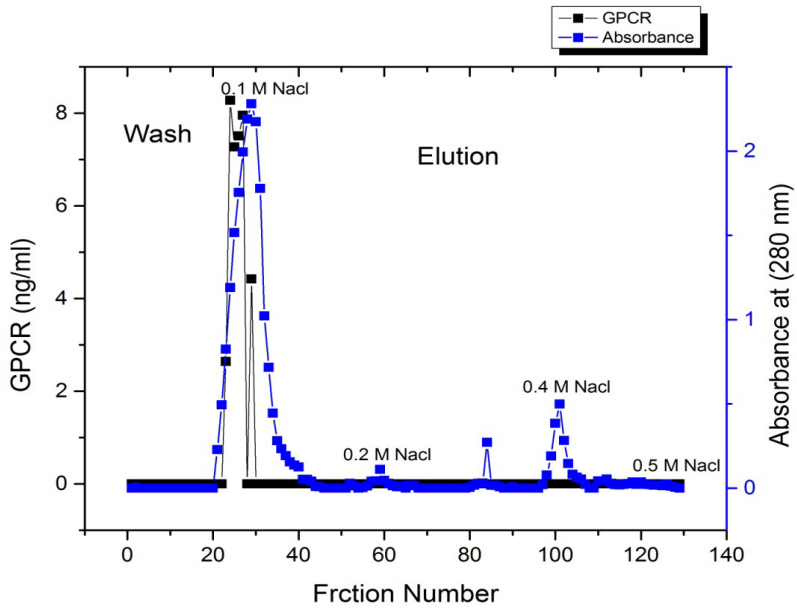
3.Results and discussion:

The local strain of *S.cerevisiae* were identified by studying specific microscopically, morphological and biochemical characterization(Kurtzman 2011) The ability of isolate *S.cerevisea* for fermentation and assimilation was examined ,which glucose, fructose, sucrose, galactose, lactose, maltose, raffinose were used. The results illustrated that glucose, fructose, sucrose, galactose, , maltose, raffinose) were fermented and assimilated by the

isolate strain while lactose was not fermented and assimilated by this isolate. The isolate also show un ability to hydrolyzed urea and produce ammonia. The characters are in agreement with previous study(Ladds 2003)

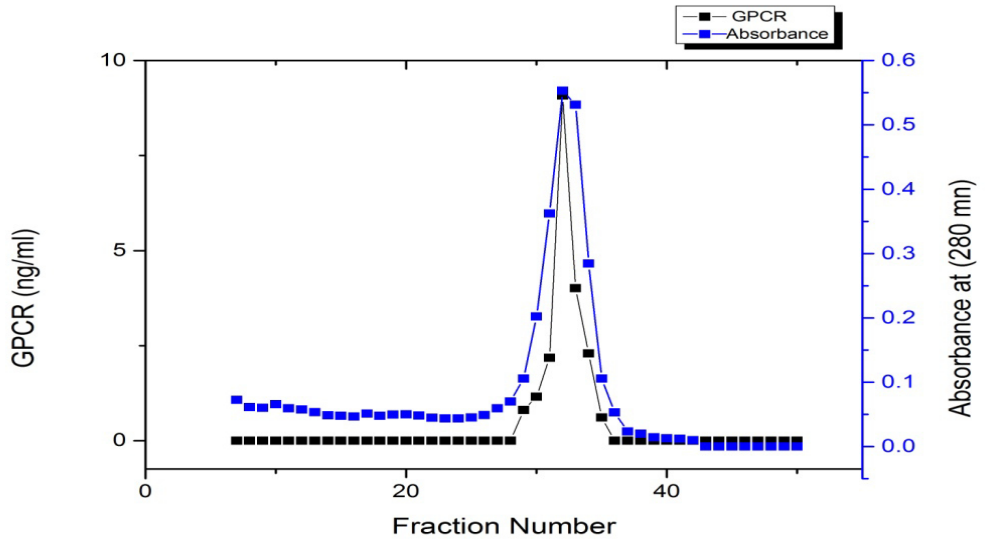
Yeast cell were thawed and resuspended in extraction buffer and disrupted the cell well with ultrasonicater .All steps performed at 4C˚and all butters were supplemented with protease inhibitors cocktail(Marris 2001).

The GPCR that extracted from whole cell of local strain of *S.cerevisiae* was purified in the first step by ion exchange chromatography using DEAE- sepharose (an anionic ion exchange) that washed and eluted by (50mM PBS +DDM) ,the fraction were collected with 5 ml tube at a flow rate 30 ml/ h and eluted with gradient (0.1-0.5M) of sodium chloride solution. Two proteins peaks appeared after elution by the gradient concentration of sodium chloride, while no protein peaks appeared in the washing fractions. The GPCR concentration was measured in the fractions of these two protein peaks, data indicated that GPCR located in the first protein peak (eluted at 0.1M of NaCl) at fraction numbers between 3 and 9 while the second peaks (eluted at 0.4 M of NaCl) don't give any concentration for GPCR , thus its neglected .



Figure(1): Purification of GPCR by Ion exchange chromatography

Fractions represent GPCR were pooled and concentrated to (5ml) by sucrose, then protein concentration, GPCR concentration were measured as shown in table (1).Gel filtration chromatography was used as second step of purification which applied by using sepharose 6B. Aliquot of five ml of concentrated fractions from step one was injected into column (1.6x 96 cm) which previously equilibrated with 50 mM phosphate buffer saline (pH= 7.4), and eluted with elution buffer with flow rate of 30 ml/h and 5ml for each fraction .Results displayed in figure (2) show single active protein peaks appeared that identical with the peak of GPCR at fractions numbers(29-35).



Figure(2):Purification of GPCR by Gel filtration chromatography

The maximum concentration of GPCR that observed in the fractions (29-35) was 9.082 (ng/ml)

The specific activity for these fractions was 151.37 (ng/mg) protein with yield of 39.64% .

The molecular weight and purity of GPCR was determined by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) as shown in the figure (3). The GPCR that extracted from whole cell was showed (~ 33 KD) with single band.

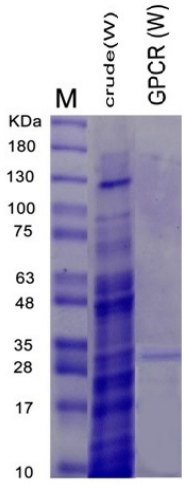


Figure (3):Polyacrylamide gel electrophoresis of purified GPCR.W: Whole cell.

Table(3.3): Volume /protein concentration, GPCR concentration , specific activity ,purification fold and yield for all purification Steps of GPCR from whole cell of locally isolated *S. cerevisiae*.

steps	Volume (ml)	Protein Conc. mg/ml)(	GPCR Conc. (ng/ml)	Specific Activity (ng/mg)	Purification Fold	Yield(%)
Crude GPCR	10	0.50	11.453	22.906	1	100
Ion exchange step	9	0.13	281.9.	71.58	3.125	72.9
Gel filtration step	5	0.06	9.082	151.37	6.608	39.64

The isolation of eukaryotic membrane proteins produced in *S. cerevisiae* is challenging, and the limited number of membrane protein structure is due to difficulties encountered with production, solubilization and purification of appropriate amounts of membrane proteins that are able to from crystals diffracting at a high resolution(Wong 2011, Clark 2010).

Another study revealed that expression does not always lead to successful functional purification (O'Malley 2003). In recent study developed a platform by using *Saccharomyces cerevisiae* for the rapid construction and evaluation of functional GPCR variants for structural studies was performed . This platform enables us to perform a screening cycle from construction to evaluation of variants within 6-7 days, we firstly confirmed the functional expression of 25 full- length class A GPCRs, then in order to improve the expression level and stability generated and evaluated the variants of the four GPCRs (HADRB2, hcHRM2, hHRHI and hNTSRI) were performed, these stabilized receptor variants improved both functional activity and monodispersity, finally the expression level of the stabilized hHRH1 in pichia pastoris was improved up to 65 pmol/ mg from negligible expression of the functional full- length receptor in S. cerevisiae at first screening. The stabilized hHRH, was able to be purified for use incrystailization trials The results also demonstrated that the S. cerevisiae system should serve as an easy- to- handle and rapid platform for the constructure and evaluation of GPCR variants. This platform can be powerful prescreening method to identify a suitable GPCR variant for crystailography (Shimamura 2011).

Other recent study demonstrated that to produce large quantities of high quality eukaryotic membrane proteins in S. cerevisiae they modified a high- copy vector was modified to express membrane proteins c-terminally – fused to a tobacco virus protease detachable(Scharaff -pouslen 2013).

The present study a chive a relatively high purification of GPCR from whole cell of a local strain *S. cerevisiae* with fold purification 6.608 and a yield of 39.64 % and molecular weight about~33KD.

As far to our knowledge this is the first study reported the purification of GPCR from local strain of S. cerevisiae in Iraq by two steps firstly, by using DEAE- sepharose in ion exchange chromatography and sepharose 6B in gel filtration chromatography.

4. Conclusion

The present study chief a relatively high purification of GPCR from *S.cerevisiae* by low cost materials used as risen .GPCR that purified from whole cell of a local strain *S. cerevisiae* are with 6.608 fold purification, 39.64 % a yield and molecular weight about~33KD.

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