Hittite Journal of Science and Engineering, 2018, 5 (4) 271-274
ISSN NUMBER: 2148-4171
DOI: 10.17350/HJSE19030000102

# Review of Chromatography Methods for Purfication of Paraoxonase Enzyme

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**ABSTRACT**

Human serum paraoxonase1 (hPON1) is a calcium dependent enzyme, which has an important physiological role in organism with detoxification, antioxidant and also antiatherogenic properties. PON1 is mostly synthesized from the liver than secreted in serum with located on HDL.

In order to better understand the molecular mechanism of paraoxonase 1, researchers have been increasingly interested in the development of various methods for obtaining the enzyme in pure form over the last decades. In this paper, several different purification and characterization methods of multi functional enzyme paraoxonase 1(EC 3.3.8.1) was reviewed.

***Article History:***
*Received: 2017/11/23*
*Accepted: 2017/12/25*
*Online: 2018/01/31*

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**Keywords:**
Paraoxonase1, Chromatography Methods, Enzyme Purification and Characterization

## INTRODUCTION

### Paraoxonase Enzyme

In humans, the paraoxonase (PON) gene family has three members, PON1, PON2 and PON3 are aligned side-by-side in the long arm. chromosome 7q21.3-22.1. Although PON3 identified as a lactonase in rabbit HDL, PON2 has not yet been found in serum, though expresses in many biological tissues. PON1, considered as a component of HDL in mammalian, metabolizes lipid peroxides and prevents against accumulation on LDL **[1-3]**.

PON1 is a glycoprotein consist of 354 amino acids that approximate molecular mass of 43 kDa and its active site involves two calcium ions, one essential for stability and the other one is essential for activity. Paraoxon substrate is widely used to measure paraoxonase enzyme activity. Furthermore, it has aryl esterase, organophosphates and lactonase activities, but the physiological substrate is still unknown **[4-5]**. PON1 is linked to HDL in circulation which has a protection against atherosclerosis, especially by protecting the oxidation of LDL lipids. The paraoxonase enzyme has an N-terminal hydrophobic region on it **[6-7]**. For this reason, generally hydrophobic interaction chromatography (HEK) was chosen for the purification of the enzyme.

PON1 is not only a nerve agent and toxic pesticide hydrolyzer but also associated with several many diseases (aging, coronary artery diseases, cancer, diabetes mellitus Type 2, obesity, neurological disorders, connective tissue disorders, gynecology, liver disorders. adrenal diseases) **[8-11]**.

### The Importance of Purifying Enzymes

With the recent developments in biotechnology, the purification of the enzyme has gained importance by increasing the usage areas and new application fields of enzymes.

A purification technique is to achieve the target protein at the lowest cost, high purity and efficiency. The purified protein has a certain purpose. For this purpose method and protein should be chosen before the separation. The protein obtained in pure form is not a finish, but a beginning for academic or industrial usage. While the degree of purity of the enzyme is important for academic research, yield is important for industrial use. These studies suggest that protein activity, structure or function relationships **[12].** With the purification, we can examine the amino acid sequences, biochemical and physiological functions properties and interaction with other metabolites, which of proteins. The target enzyme or receptor can be purified by chromatography method, which is a chromatographic purification technique by directing it on a substrate, ligand or a substrate analog.

**Purification of Paraoxonase Enzyme**

The purification procedures of paraoxonase 1 have been described mostly at the beginning of the 90's.

Besides ApoA-I, the primary established apoprotein constituent, a number of proteins can bind paraoxonase-bearing HDL as well. To get rid of plate contamination HDL-related proteins such as apoproteins B, C, D, E, and ghrelin have been identified as susceptible to contamination of paraoxonase1 samples **[13]**. In literature there are different protocols and different sources have been used for purification and characterization of paraoxonase enzyme **[14-27]**.

Furlong and colleagues have shown that PON enzyme purified 62.1 fold of human serum by four step purification protocols consisting of Agarose Blue, Sephadex G-200, DEAE-Tris acryl M and Sephadex G-75 **[14]**. Gan et al. have founded that the enzyme isoelectric point is 5.1 and the molecular weight of the enzyme is 43 kDa by purifying the enzyme paraoxonase **[15]**.

Rodrigo et al. purified paraoxonase for the first time from rat liver 415-fold and specific activity of 1370 units/ mg with five steps protocols **[16]**. The enzyme was purified from sheep liver microcosm by three step procedures including % 20- % 70 ammonium sulfate precipitation, DEAE-Sephadex A50 anion EC and Sephadex G-100 gel FC with nearly 141-fold but very low yield % 3,5 [**17**]. Beydemir et al. showed that PON1 was purified 225 times by ion exchange then gel filtration chromatography **[18]**.

On account of reducing the number of purification steps and increase yield of the paraoxonase enzyme, researchers have focused to develop novel purification method, especially HIC by taking advantage of the hydrophobic character of the enzyme.

**Purification of Paraoxonase Enzyme by HIC**

Hydrophobic interaction chromatography separates proteins by their hydrophobicity differences. The separation method is based on a protein between a reversible interaction with the hydrophobic surface of a chromatographic medium. Hydrophobic interactions are one of the greatest forces that provide the stability of three-dimensional structures of protein molecules and most biomolecules exhibit a certain degree of hydrophobic character. Hydrophobic interactions play a role in antigen-antibody, enzyme-substrate reactions, formation of the lipid bilayer and binding of the protein molecules to the membrane. For the purification of protein molecules according to HIC; the tertiary and quaternary structure of proteins which are the source of surface hydrophobicity are utilized. It is necessary to distinguish this from the hydrophobicity difference arising from the primer structure of the protein. In HIC, the separation by which the hydrophobic regions on the surface of the protein molecules are utilized is carried out under conditions in which the integrity of the protein molecules is preserved.

Sinan et al. **[19]** purified human paraoxonase including two-step procedures, first ammonium sulfate precipitation and then novel synthesized hydrophobic interaction chromatography called Sepharose-4B-L-tyrosine-1-naphthylamine. Overall purification rate was found 227-fold and 72,54 % yield. Before loading in the column; the precipitate was saturated with 1 M $(NH_4)_2SO_4$ to enhance the coupling efficiency of the hydrophobic interaction chromatography column. Above-mentioned hydrophobic gel was synthesized by diazotization of 1-naphthylamine and its binding to Sepharose-4B and L-tyrosine respectively **[19]**.

This straightforward hydrophobic interaction chromatographic protocol including two step procedure has been used by many investigators for the purification and characterizing of the paraoxonase enzyme from different sources shown in **Table 1 [20-22].**

Sayın et al. **(20)** purified Paraoxonase firstly was shark *Scyliorhinus canicula* serum by using the same method. The overall purification rates were 37-fold with a yield of 127%. This work has disproved the thesis that the enzyme paraxonase is not found in fish. **[20]**. It was reported that paraoxonase 1 was purified and characterization of Swiss Black, Holstein, and Montofon Bovine's serums via first (%60-80) ammonium sulfate precipitation then Sepharose-4B-L-tyrosine-1-naphthylamine with 262.47-, 2,476.90-, and 538.06-fold for Swiss Black, Holstein, and Montofon bovines, respectively **[21]**. In a different study as enzyme sources were choosed from Kivircik and Merinosheep's serums for purification characterization of PON enzyme with Sinan's method **[19]**. Km values were found 0.482 mM and 0.153 mM, Vmax values were found 41.348 U/mL.min and 70.289 U/mL.min for MerinoPON, and KivircikPON respectively **[22]**.

Various modified hydrophobic interaction chromatography methods are available in the literature, using different extension arms and different pre-purification processes shown in **Table 1 [23-24]**. Ekinci et al. applied three-step procedures. After ammonium sulfate precipitation, diethylaminoethyl-Sephadex AEC and newly synthesized Sepharose 4B-4-phenylazo-2-naphthaleneamine hydro-phobic interaction chromatography were applied to purify the enzyme with 302-fold and yield of 32% **[23]**. In another study, firstly serum was treated with Triton X-100 then the enzyme was purified with Sepharose-4B-aniline-2-naphthylamine gel with 356 purification fold times by researchers [24].

**Table 1: Isolation of paraoxonase (E.**C. 4.2.1.1) enzyme by using hydrophobic gels.

| *Enzyme source* | *pre-purification processes* | *Ligand* | *Spacer arms* | *Overall purification rate* |
|---|---|---|---|---|
| *Human Serum* | *Ammonium sulfate precipitation (ASP)* | *1- naphthylamine* | *L-tyrosine* | *227-fold 72,54 % yield (19).* |
| *Scyliorhinus canicula serum* | *ASP* | *1- naphthylamine* | *L-tyrosine* | *37-fold 127 % yield (20).* |
| *Swiss Black, Holstein, and Montofon Bovine's serums* | *ASP* | *1-naphthylamine* | *L-tyrosine* | *262.47, 2,476.90, and 538.06 fold respectively (21).* |
| *Merino Sheep's Serum and Kıvırcık Sheep's Serum* | *ASP* | *1-naphthylamine* | *L-tyrosine* | *462.7 fold with % 6.5 yield and 461.7 fold with % 6.4 yield (22).* |
| *Human Serum* | *Triton X-100–treated serum ASP IEC* | *2-naphthaleneamine* | *4-phenylazo-* | *302 fold % 32 yield (23).* |
| *Human Serum* | *Triton X-100–treated serum ASP IEC* | *2-naphthaleneamine* | *aniline* | *356 fold (24).* |
| *Human Serum* | *ASP* | *9-aminophenanthrene* | *L-tyrosine* | *526 fold and % 39,8 yield (25).* |
| *Human Serum* | *ASP* | *3-aminophenantrene* | *L-tyrosine* | *219 fold and % 10 yield (26).* |
| *Human Serum* | *ASP* | *1-aminophenantrene* | *L-tyrosine* | *674 fold and % 16 yield (27).* |

In the last decades, researchers have focused on the effects of new ligands posses different hydrophobic characteristics on the purification of paraoxonase 1 enzyme shown in **Table 1 [25-27].** For this purpose, alternative novel HIC gels were synthesized. Amino anthracene and amino phenanthrene have three cyclic aromatic ring and more hydrophobic from naphthylamine. Gencer et al. **[27]** were used various ligands where amine was attached to different numbered carbons on **[25-27]**. In one study as ligand 9-aminophenanthrene was conducted. Since this ligand has more hydrophobic character than 2-naphthylamine, PON1 enzyme was attached HIC column tightly. According to this method purification fold was obtain 526 times, but yield 39,8 % **[25]**, and the yield value is lower than the of Sinan et al.'s method. The large hydrophobic character for ligand selection in HIC can be disadvantageous. Because the hydrophobic interactions will be stronger, the elution of the enzyme may be difficult. Demir et al, conducted an alternative hydrophobic interaction chromatography gel namely, Sepharose-4B-L-tyrosine-3-aminophenantrene. In this study researchers show that hPON1 was purified 219-fold and very low yield with of 10%. Also the effects of some anabolic steroid compounds on pure enzyme were investigated as a drug target study **[26]**. Gençer et al. **[27]** developed a novel HIC method include ammonium sulfate precipitation and Sepharose-4B-L-tyrosine-1-aminoanthracene. Enzym purity was controlled by SDS-electrophoresis with a single band on 43kDa molecular weight. As well, both purification factor and yield of hPON1 were calculated 674-fold and 16 % respectively (27). The biggest problem came across is that the proteins can be absorbed very efficiently; the elution is usually a distressing condition due to the strong bonding in hydrophobic interaction chromatography. Even though a like difficulty could be experienced in affinity chromatography, hydrophobic interaction chromatography should be take into account in any general optimization procedure.

## CONCLUSION

In the last few decades, studies have been increasingly focused on the physiological and biological properties of the paraoxonase enzyme. Paraoxonase act as an ideal drug target molecule for many diseases that both being connected to the HDL and possessing strong antioxidant properties. But studies in which the enzyme paraoxonase is used as a drug target are still very limited. Structural and functional researches require high purity enzyme, but for PON1, purity is a challenge work. The particular HDL environment of multiple interacting lipids and proteins may explain why study of paraoxonase in solution is so hard. The three-dimensional (3D) structure of human PON1, the catalytic mechanism and the physiological substrate of PON1 are still uncertain. In addition the drawbacks of this multifunctional enzyme are both not commercially available and still not an affinity chromatography method. These are reasons why purification method of paraoxonase must be well carried out. Here, the purification procedures of multifunctional molecule paraoxonase enzyme are thoroughly reviewed from the past to the present.

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