Asian Journal of Chemistry; Vol. 23, No. 8 (2011), 3657-3659

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Effect of Ammonium Sulphate on the Activities of Paraoxonase Isoenzymes Q and R

NAHIT GENÇER* and OKTAY ARSLAN

Department of Chemistry, Faculty of Science and Art, Balikesir University, 10145 Balikesir, Turkey

*Corresponding author: Tel: 90 266 6121278; E-mail: ngencer@balikesir.edu.tr

(*Received*: 26 November 2010; *Accepted*: 27 April 2011) AJC-9870

Paraoxonase isoenzymes exists in 2 polymorphic forms, Q (glutamine) or R (arginine) at codon 192. The Q isoform has a higher activity for hydrolyze of diazoxon, sarin and soman; whereas the R isoform has a higher activity for hydrolyze of paraoxon and chlorpyrifosoxon. In this study the effect of ammonium sulphate was investigated on the Q and R isoenzymes. For this purpose, ammonium sulphate precipitation was performed before the Q and R isoenzymes. After ammonium sulphate precipitation the specific activity of R isoform is 20.7 mU/mg. However, after ammonium sulphate precipitation the specific activity of Q isoform is 6.6 mU/mg. After dialysis the specific activity of R isoform is 6.3 mU/mg. However, after ammonium sulphate precipitation the specific activity of Q isoform is 4.8 mU/mg. Q isoenzymes activity did not change significantly as a result of these applications. However, the R isoenzyme activity increased before and decreased in post-dialysis. Ammonium sulfate was found to stimulate the R isoenzyme. This enzyme can use at immobilization and chromatographic procedures; a very important activity is high. If the first step, ammonium sulphate precipitation is performed by selecting the R isoenzyme activity rather increased. Then, the high activity of enzyme can be used easily with other purification methods and immobilization studies.

Key Words: Paraoxonase, Ammonium sulfate, Polymorphism.

INTRODUCTION

Paraoxonase (EC 3.1.8.1, PON1) is a calcium dependent serum esterase that is synthesized by the liver. In serum, it is closely associated with high-density lipoproteins[1,2]. Paraoxonase hydrolyze organophosphate compounds are widely used as insecticides and nerve gases. Therefore, PON1 plays a major role in the detoxification of these compounds and other artificial substrates, so that it may alter significantly an individual's susceptibility to the toxicity of these chemicals. In addition, paraoxonase is involved in lipid metabolism, since this enzyme probably hydrolyzes multiple oxygenated forms of polyunsaturated fatty acids of low-density lipoproteins associated with phospholipids. For this reason, paraoxonase can be defined as an antioxidant enzyme[3,4].

PON1 contains two major polymorphisms as the result of amino acid substitution at position 55 (leucine *versus* methionine) and at position 192 (glutamine: Q *versus* arginine: R)[5,6]. The PON1192 activity polymorphisms are substrate dependent. The PON1Q192 isoform has a higher rate of *in vitro* hydrolysis of diazoxon, sarin and soman[7], whereas the PON1R192 isoform has a higher activity for hydrolysis of paraoxon and chloropyrifos oxon[8]. In addition the ability of HDL to protect LDL against peroxidation *in vitro* is significantly lower in HDL particles containing PON1R192 than in those with PON1Q192[9].

The activity of paraoxonase enzyme in serum is very low. Various methods are used for its purification from human serum. Methods involved in its purification are elaborate and complicated. Also the yield and final activity are highly variable. This enzyme is usually purified by chromatographic methods. Purification step increases, enzyme activity gradually decreased. This study investigated the effect of ammonium sulphate on the Q and R isoenzymes. This study is the first study to examine the effect of ammonium sulfate, Q and R isoenzymes. For this purpose, ammonium sulphate precipitation was performed before the Q and R isoenzymes, more then subjected to the process of dialysis. Q isoenzymes activity did not change significantly as a result of these applications. However, the R isoenzyme activity increased before and decreased in post-dialysis. Ammonium sulfate was found to stimulate the R isoenzyme. This enzyme can use at immobilization and chromatographic procedures. If the first step, ammonium sulphate precipitation is performed by selecting the R isoenzyme activity rather increased. Then, the high activity of enzyme can be used easily with other purification methods and at immobilizations. In this study, ammonium sulphate precipitation was applied before the R and Q isoenzymes. Then, each isoenzyme was performed in dialysis.

Initially and after each stage of activity and protein amounts were determined.

EXPERIMENTAL

The materials used include, paraoxon, protein assay reagents and chemicals were obtained from Sigma Chem. Co. All other chemicals used were analytical grade.

Phenotyping and purification of human PON1 Q and R types: In order to classify individual phenotypes, two parameters were used. According to Eckerson *et al.*[10], phenotypic distribution of the paraoxonase activity was determined by the basal and stimulation of paraoxonase activity by 1M NaCl. Individuals were classified for paraoxonase phenotype using the antimode at 60 % stimulation as the dividing point between the non-salt-stimulated, Q type and the salt-stimulated, QR (60-200 %) and R (200 %-up) types.

Ammonium sulphate precipitation: Human serum was isolated from fresh human blood taken to dry tube. The blood samples were centrifuged at 1500 rpm for 15 min and the serum was removed. Firstly, serum paraoxonase was isolated by ammonium sulphate precipitation (60-80 %)[11]. The precipitate was collected by centrifugation at 15000 rpm for 20 min and redissolved in 100 mM *tris*-HCl buffer (pH 8.0).

Dialysis: The diluted protein fractions from ammonium sulphate precipitation were pooled and kept for dialysis in a dialysis bag with 8 KDa cut-off. Dialysis was carried out overnight against double distilled water with 2 changes. This process facilitates removal of ammonium sulphate which was used previous step. Long time association of ammonium sulphate with paraoxonase affects its activity as well as its half life.

Total protein determination: The absorbance at 280 nm was used to monitor the protein in the column effluents and ammonium sulfate precipitation. Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford[12], with bovine serum albumin standard.

Paraoxonase enzyme assay: Paraoxonase enzyme activity towards paraoxon was quantified spectrophotometrically by the method described by Gan *et al.*[13]. The reaction was followed for 2 min at 37 ºC by monitoring the appearance of *p*-nitrophenol at 412 nm in Biotek automated recording spectrophotometer. A molar extinction coefficient of *p*-nitrophenol at pH 8.0 in 100 mM *tris*-base buffer of 17,100 M^{-1} cm^{-1} was used for the calculation. PON1 activity (1 U L^{-1}) was defined as 1 μmol of *p*-nitrophenol formed per minute.

RESULTS AND DISCUSSION

Paraoxonase is a complex enzyme and its physiological role has not been clarified since no natural substrates of this enzyme are known. Interestingly, in addition to its role in lipid metabolism, cardiovascular disease and atherosclerosis. PON1 plays a role in the metabolism of pharmaceutical drugs[14]. Polymorphism of the PON1 gene effects the blood levels PON1 and its catalytic efficiency; both factors strongly effect an individual's susceptible to arteriosclerosis, pollutants and insecticides[15,16]. In addition, it was supported the evidence of that mice lacking PON1 are highly susceptible to arteriosclerosis and organophosphates poisoning[16]. In order to classify individual phenotypes, two parameters were used. According to Eckerson *et al.*[10], phenotypic distribution of the paraoxonase activity was determined by the basal and stimulation of paraoxonase activity by 1M NaCl.

This study investigated the effect of ammonium sulphate on the Q and R isoenzymes. For this purpose, ammonium sulphate precipitation was performed before the Q and R isoenzymes, more then subjected to the process of dialysis. Q isoenzymes activity did not change significantly as a result of these applications. However, the R isoenzyme activity increased before and decreased in post-dialysis. Table-1 shows the typical enzyme activity for R and Q isoform. The enzyme activity and total protein concentration were determined from all fractions collected from each step. After ammonium sulphate precipitation the specific activity of R isoform is 20.7 mU/mg. However, after ammonium sulphate precipitation the specific activity of Q isoform is 6.6 mU/mg. After dialysis the specific activity of R isoform is 6.3 mU/mg. However, after ammonium sulphate precipitation the specific activity of Q isoform is 4.8 mU/mg. Ammonium sulfate was found to stimulate the R isoenzyme. This enzyme immobilization and chromatographic procedures are used; a very important activity is high. If the first step, ammonium sulphate precipitation is performed by selecting the R isoenzyme activity rather increased. Then, the high activity of enzyme can be used easily with other purification methods.

ACKNOWLEDGEMENTS

Special thanks to Dr. Selma Sinan for her help and advice during the study.

TABLE-1
SCHEME FOR PURIFICATION OF Q AND R ISOENZYME ACTIVITY

Fraction	Volume (mL)	Activity (U/mL)	Total activity (U/mL)	Protein amount (mg/mL)	Total protein (mg/mL)	Specific activity (mU/mg)
Q type						
Serum	20.0	37.9	758.0	7.5	150.0	5.0
Ammonium sulphate precipitation	8.3	40.2	333.7	6.1	50.6	6.6
Dialysis	8.2	29.4	241.1	6.1	50.0	4.8
R type						
Serum	20.0	38.1	826.0	7.5	150.0	5.5
Ammonium sulphate precipitation	8.3	126.1	1046.6	6.1	50.6	20.7
Dialysis	8.2	38.4	314.9	6.1	50.0	6.3

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