Peroxiredoxin(PRX) gene family characterization in aves

Sang Su Shin[1], Tae Min Kim[1], Ji Hye Shin[3], Tae Sub Park[1,2], Jin-Kyoo Kim[4], Heebal Kim[1] and Jae Yong Han[1]

[1]Division of Animal Genetic Engineering, School of Agricultural Biotechnology, [2]Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea

[3]Avicore Biotechnology Institute Inc., Hanlim Human Tower #707, Geumjeong-Dong 1-40, Gunpo city, Kyonggi-Do 435-050, Korea

[4]Department of Microbiology, College of Natural Sciences, Changwon National University, Changwon 641-773, Korea

Abstract

Peroxiredoxin(PRX)은 원핵세포에서 진핵세포에 이르기까지 세포 내부적으로 발생된 과산화물로부터 자신을 보호하는 중요한 항산화단백질이다. 포유류에서는 아직까지 여섯 개의 다른 동형체가 밝혀졌으며, 조류에서는 아직 발표된 바가 없다. 이 실험을 통해 최초로 조류의 PRX 단백질군들의 특성을 분석하였다. 생물정보분석기법을 통해 알아본 결과, 조류에서는 최소한 진화적으로 보존된 4개의 다른 PRX 단백질로 구성됨을 알 수 있다. 또한 닭의 PRXs로 in vitro 실험을 진행한 결과, 포유류의 것과 비슷한 항산화 활성을 나타냄을 알 수 있었다.

닭의 PRX는 조직 비특이적으로 발현하였으며, 이는 항산화 물질의 피해로부터 모든 조직을 보호하기 위한 필수적 요소이기 때문일 것으로 추정된다. 결론적으로, 생물정보분석기법을 통하여 추정할 수 있는 닭의 기능성 유전자군을 효과적으로 찾을 수 있고, in vitro 실험을 통하여 그 기능을 확인할 수 있었다.

Key words : chicken, oxidative damage, antioxidant activity, PRXs

Introduction

Peroxiredoxins(PRXs) are a family of thiol-containing peroxidase without any prosthetic group. The PRXs are a major antioxidant of endogenously-produced peroxides in eukaryotes, and they are conserved from bacteria to mammals. The PRXs are divided into three classes and all PRXs share the same basic catalytic mechanism, in which an active-site cysteine is oxidized to a sulfenic acid by the peroxide substrate(Wood et al., 2003).

Now, six different isoforms of PRXs have been identified in mammals(Fujii and Ikeda, 2002) and they are involved not only oxidative stress protection mechanisms but also cell differentiation, proliferation, immune response, and apoptosis (Yuan et al., 2004). Here, we characterized the *PRX* genes, their antioxidant activity and expression pattern in the chicken.

Materials and Methods

Database search and domain analysis were conducted by nr protein database of NCBI, GgGI of TIGR, ESTScan and PSI-blast programs, and HMMER and Pfam-A family matrices. ClustalW program and NEIGHBOR were used to phylogenetic analysis. Chicken *PRX*(*chPRX*) *I* and *VI* cDNAs were subcloned into the bacterial expression vector, pET21b(Novagen), fused to His-tag

and expressed in *E. coli* BL21 (DE3). And its molecular weight and metal-catalysed oxidation (MCO) activity were determined.

Finally, the expression pattern was studied in different chicken tissues (brain, intestine, liver, testis, and sple) by RT-PCR with *chPRX I* primers.

Results

Through the database search and domain analysis, 19 putative PRX proteins are identified and they were collapsed into four different locations of the chicken genome sequences indicating that four different genes represent the 19 alternative splicing variants. And the phylogenetic analysis showed that at least four different kinds of PRX protein (PRX Ⅰ, Ⅲ, Ⅳ and Ⅵ) are conserved in aves over its evolutionary lineage. The putative chicken *PRXI* and *VI* were cloned by RT-PCR and expression vectors for *E. coli* were designed.

They were expressed effectively and the molecular weights were approximately 23 kDa and 25 kDa, respectively. The purified recombinant chicken PRX I (rchPRX I) protein could successfully protect circular plasmid DNA from dithiothreitol (DTT) and FeCl3 reagents and this activity of chicken PRX protein was similar to that of mammals.

The expression patterns of *PRX I* or *VI* in the chicken tissues showed that both *chPRXs* were constantly transcribed in all of the tested tissues. This result suggested that *chPRXs* might be housekeeping gene involved in the repairing the oxidant damage for homeostasis. Although we did not find out any new function of this proteins, we characterize the *PRX* genes, their antioxidant activity and expression pattern in the chicken.

Acknowledgement

This work was granted from BioGreen 21 program, Rural Development Administration, Republic of Korea and also supported by a graduate fellowship from the Brain Korea 21 project of the Korean Ministry of Education.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST : a new generation of protein database search programs. Nucleic Acids Res. 25, 3389-3402.

Fujii, J., Ikeda, Y., 2002. Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. Redox. Rep. 7,123-130.

Wood, Z.A., Schroder, E., Robin Harris J., Poole, L.B., 2003. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem. Sci. 28, 32-40.