

# Thimerosal stimulates focal adhesion kinase and cytoskeletal changes by redox modulation

Euikyung Kim, Jung Hwan Kim, Hyeon Soo Kim, Sung Ho Ryu, Pann-Ghill Suh\*

*POSTECH Department of Life Science, Division of Molecular and Life Science, Pohang University of Science and Technology, San 31, Hyoja-Dong Nam-Gu, Pohang, Kyungbuk 790-784, South Korea*

Received 22 March 2002; received in revised form 8 July 2002; accepted 11 July 2002

## Abstract

Thimerosal is one of the most widely used preservatives and has been reported to cause chemically mediated side effects. However, the mechanism of the side effects is not clearly understood yet. In the present study, we showed that HeLa S cells treated by thimerosal generated reactive oxygen species (ROS). Thimerosal-generated ROS stimulated the tyrosine phosphorylation of focal adhesion kinase (FAK) and also induced cytoskeletal changes. Pretreatment with intracellular calcium chelator, BAPTA did not block the thimerosal-mediated FAK tyrosine phosphorylation. On the other hand, either FAK inhibitor, tyrphostin or ROS scavenger, *N*-acetyl-L-cysteine (NAC) suppressed the tyrosine phosphorylation and cytoskeletal changes. These results suggest that thimerosal seems to induce FAK tyrosine phosphorylation and cytoskeletal changes by ROS generation but not by intracellular calcium mobilization. We think the present finding can be an important clue to understanding the mechanism of thimerosal-mediated side effects, such as contact dermatitis, and allergy.

© 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Focal adhesion kinase; Tyrosine phosphorylation; Cytoskeletal change; Thimerosal; Reactive oxygen species

## 1. Introduction

The antibacterial and antifungal activity of thimerosal has been used as preservatives for various biological products, including vaccines, cleaning solutions for eye lenses, as well as cosmetics. The wide use of thimerosal in biological materials has often resulted in public health issues by causing thimerosal-mediated side effects such as contact dermatitis, and inflammatory responses [1,2]. Thimerosal in cleaning solutions for eye lenses has been previously shown to produce cytotoxicity for corneal epithelial cells [3–5]. The molecular mechanism of the side effects, however, has not been clearly described yet. The most well-characterized biological activity of thimerosal is the intracellular calcium mobilization by thimerosal, which has been previously manifested in many different cell types, such as smooth muscle cells, endothelial cells, HeLa cells, platelets, neutrophils, lymphocytes, and so on [6]. Thimerosal is a thiosalicylic acid derivative containing ethyl mercury (Fig. 1A) and

the mercury atom is essential for the calcium mobilizing activity of the compound. Thiosalicylic acid, a thimerosal structural analogue, which does not contain the ethyl mercury, has no calcium releasing activity. Furthermore, the mercury atom of thimerosal gives the compound an oxidative character and some protein sulfhydryl groups, such as ATPase  $\text{Ca}^{2+}$  pump of the sarcoplasmic reticulum, are reported to be redox modulated by thimerosal [7]. In this study, we suggest that focal adhesion kinase (FAK) is one of the important target molecules of thimerosal.

FAK is a nonreceptor protein tyrosine kinase (PTK) and a key mediator of integrin signaling, which implicates its regulatory roles in cell adhesion, spreading, migration as well as cell survival and proliferation. Stimulation of FAK tyrosine phosphorylation has been reported in many different cell types by various kinds of stimuli, which can be integrin-independent or integrin-dependent [8]. Upon stimulation, FAK can be autophosphorylated on tyrosine 397, recruiting other nonreceptor PTKs, pp60src and pp59fyn, via their SH2 domains [9], which can create additional tyrosine phosphorylation on other residues of FAK. As one of the integrin-independent signals,  $\text{H}_2\text{O}_2$  has been reported to induce FAK tyrosine phosphorylation in vascu-

\* Corresponding author. Tel.: +82-54-279-2293; fax: +82-54-283-4613.  
E-mail address: pgs@postech.ac.kr (P.-G. Suh).