

EXPANDED ABSTRACT

Preliminary study of modification of serum albumin through paracellular pathway of rat submandibular gland

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INTRODUCTION

One of structural characteristics in human serum albumin is a single reactive free sulfhydryl group in position 34. In extracellular fluid such as serum and interstitial fluid, albumin is a mixture of oxidized forms and reduced form having free sulfhydryl group (1). The oxidized form of albumin is induced by oxidation of free sulfhydryl group due to several oxidants such as cystine, oxidized glutathione and hydrogen peroxide. It has been reported that the sulfhydryl group in albumin is highly oxidized in aging, exercise and renal dysfunctions, having attracted clinical application as an extracellular marker of oxidative stress (2-4). However, the oxidization mechanism including the site within organ still remains to be explained. The isolated rat salivary gland (5) allows us to sample venous effluent from the organ, interstitial fluid and saliva, thus useful for understanding the site of oxidization through capillary, across vascular wall or through paracellular route. In this study, to clarify the oxidization mechanism of albumin in the living system, we isolated a rat submandibular gland and perfused it vascularly with the perfusate containing commercial human albumin. Then, the collected samples were analyzed by a specially designed system of high-performance

liquid chromatography (HPLC) which could separate albumin into reduced and oxidized forms (2, 3).

MATERIALS AND METHODS

The isolation and perfusion of rat submandibular gland was followed with the procedure of Murakami, *et al.* (5). The isolated gland was perfused with HEPES buffered solution containing 2% commercial human albumin and a secretory stimulant (carbachol) for secretion of saliva. After onset of secretory stimulation, venous effluent, interstitial fluid and saliva were collected to microcentrifuge tubes. Each sample was immediately stored at -80°C until HPLC measurements. HPLC measurements were carried out by solvent gradient elution with increasing ethanol concentration from 0 to 10% in 0.05 M sodium acetate-0.40 M sodium sulfate (pH 4.85) by using of an anion-exchange HPLC column. Samples were injected to the HPLC system without chemical modification for detection of reduced and oxidized forms of albumin.

RESULTS AND DISCUSSION

Human albumins were able to detect in venous effluent, interstitial fluids and saliva from isolated rat submandibular gland. Under 1.0 µM carbachol stimulation during 60 min, the concentrations of total albumin in venous effluent, interstitial fluids, and saliva were 315.5±2.5, 195.1±12.7 and 1.0±

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0.3 μM , respectively ($n=5$). The concentration in saliva was approximately 0.05-0.7% of albumin in the perfusate. Despite the evidence that the rat salivary gland has no intrinsic secretion system of human albumin, small amount of the albumin in saliva was detected. Additionally, variation in the concentrations was large, which indicated that albumin concentration in saliva might depend on individual differences in rat. These facts suggested that the human albumin in saliva might be passed through the paracellular pathway such as tight junction in rat submandibular gland. In this study, albumin could be separated to three fractions of reduced form, reversible oxidized form and irreversible oxidized form. In time course in saliva secretion by 20 min, concentrations of total albumin in saliva were 0.7 ± 0.2 , 0.5 ± 0.1 and 0.7 ± 0.2 μM during 0-20 min, 20-40 min and 40-60 min from onset of carbachol stimulation, respectively ($n=10$). Redox states in albumin in the same time course were measured. A fraction of irreversible oxidized form which was mixed with sulfenic acid, sulfinic acid and sulfonic acid, was increased as compared with same form of albumin in the perfusate. The irreversible oxidized forms were 9.5 ± 2.1 , 15.6 ± 4.1 and $13.5 \pm 3.6\%$ of total albumin in saliva during 0-20, 20-40 and 40-60 min from onset of 1.0 μM carbachol stimulation, respectively ($n=10$). The well known pathway for sulfenic acid formation is the reaction of a thiol with oxidants such as hydrogen peroxide and peroxy-nitrite (6). In this study, increase of the oxidized form which was mixed with sulfenic acid, sulfinic acid and sulfonic acid suggested that reactive oxygen species such as hydrogen peroxide oxidized directly free sulfhydryl group of albumin in intercellular spaces in salivary gland. In conclusion, several evidences strongly suggested that the human albumin in saliva might be passed through tight junction in the

rat submandibular gland and oxidative modification of the albumin might be occurred through the paracellular pathway.

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