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Short Communication

Inhibitors of Thermally Induced Burn Incidents

Anna Pielesz*

Department of Civil and Environmental Engineering, University of Bielsko-Biala, Poland

***Corresponding author**
Anna Pielesz, Institute of Textile Engineering and Polymer Materials, Faculty of Materials and Environment Science, University of Bielsko-Biala, Bielsko-Biała, Poland, Email: apielesz@ath.bielsko.pl

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Keywords

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Abstract

In the current researches microbiological procedure, cellulose acetate electrophoresis, Fourier-transform infrared spectrometry and Scanning electron microscopy were all carried out after heating samples of chicken skin to a temperature simulating a burn incident and stimulate the release of Heat Shock Proteins. The objective of this study was to monitor the effect of temperature on collagen from organic chicken skins, which was used as a model of ex-vivo burn injured skin. Aggregates of smaller molecular weight, probably Heat Shock Proteins 37 (HSP37) proteins which undergo atrophy in presence of hydro gels like H4SiO4 × nH2O or L-Ascorbic Acid solution, were isolated by cellulose acetate electrophoresis. The examples of FTIR spectra were recorded from frozen samples of thermally modified serum.

INTRODUCTION

Animal models

The authors tried to evaluate burn wound by using an *ex-vivo* burn injured model. The objective of this study was to monitor the effect of temperature on collagen from organic chicken skins, we have used a model in which chicken skin is exposed to a variety of thermal injuries in the presence and absence of human serum albumin. The analyses were carried out after heating the samples to a temperature simulating a burn incident [1].

Preparation of organic chicken skin samples

Samples of organic chicken skin were collected, washed thoroughly and stored at deep freezer until used. First, they were washed twice in 10 wt. % of NaCl solutions to remove unnecessary proteins on their surface by stirring the solution for 48h. Then, the skins were extracted with 0.4M HCl and then with 0.5M acetic acid, the extracts were centrifuged, washed thoroughly and stored at deep freezer until used.

Bioanalytics techniques

In the current researches microbiological procedure, cellulose acetate electrophoresis (CAE), Fourier-transform infrared spectrometry (FTIR) and Scanning electron microscopy (SEM) and were all carried out after heating samples of chicken skin to a temperature simulating a burn incident.

Microbiological procedure

The samples of organic chicken skin were examined. They were exposed to bacteria that can cause nosocomial infections, that is the Gram-positive *S. aureus* and Gram-negative *E. coli*. The suspensions were transferred onto enriched agar; then, using a cooled sterile bacteria spreader, they were spread all over the agar surface and a part of skin was placed in the centre of the Petri plate. The samples of organic chicken skin were exposed to the same bacteria: *E. coli* on Mac Conkey agar (containing salts of bile acids and crystal violet inhibiting the growth of Gram-positive bacteria) and *S. aureus* on mannitol salt agar (with high concentration of NaCl inhibiting the growth of other bacteria). The samples were kept in a laboratory heater at 37°C for 24h.

CAE -Electrophoretic analysis

The samples were subjected to electrophoresis on a strip of cellulose acetate membrane (CASYS-MINI) in barbital buffer (pH 8.6) at 6mA, maximum 200V for 0.5h. The strips were stained with toluidine blue in acetic acid and then rinsed in distilled water and air-dried. The strips were stained with amido black, then rinsed and air-dried. Semi-quantitative analysis of the protein content in the samples was also conducted.

FTIR spectroscopic analysis

FTIR spectroscopic analysis was performed using a Nicolet 6700 Fourier-transform spectrophotometer with OMNIC 7.0 software and equipped on diffusion accessory Easi Diff (spectral region: 4000–500cm^{-1}, resolution: 4cm^{-1}, number of scans: 160) of the solid samples (fragments of the samples of pure organic chicken skin). Spectra of three repacked subsamples of each individual sample were averaged to one spectrum; all spectra were performed using a linear baseline and preprocessed with the Fourier smoothing (Grams 32 AI software, Galactic Industries).

SEM -Scanning electron microscopy analysis

Chicken skin surface was examined using a JSM 5500LV scanning electron microscope supplied by JEOL. The samples were mounted on aluminum stubs and coated with gold. Secondary electrons (SE) and back-scattered electrons (BSE) observations were conducted, with the accelerating voltage of 10kV.

Analysis of drugs in biological fluids - inhibitors of thermally induced burn incidents

The purpose of the study was analysed the effect of antioxidants [2]: oat, oatmeal, furoxin (dietary supplement), cranberry juice, reference β-glucan, baker's yeast, fucoidan from the dried algae from brown seaweed and bladder wrack (*Fucus vesiculosus L),* flame-retardant cyclic organophosphates and phosphonates, L-Ascorbic Acid, hydrogels like $H_4SiO_4 \times nH_2O$ on organic chicken skin changed by a burn incident and biological fluids.

Non-enzymatic glycation (Maillard reaction) *in vitro* could be a simple method to obtain glycoconjugates for studying their biological properties. The relation between the conformational properties of albumin and intermolecular interactions under effect of temperature has been the object of several biophysical studies. Also under *in vitro* conditions at 100°C – simulating a sudden burn incident – for example fucoidan binds with collagen as a result of the Maillard reaction [3].

Fucoidan from brown seaweed and flame-retardant cyclic organophosphates and phosphonates probably bind with the collagen changed by a burn incident forming a polymer film with collagen of chicken skin surface (SEM analysis), decrease the process of aggregation and recovery of native collagen [4,5]. Microbiological procedure, CAE, FTIR and SEM were all carried out after heating samples of chicken skin to a temperature simulating a burn incident and stimulates the release of HSPs. Aggregates of smaller molecular weight, probably HSP37 proteins, were isolated by cellulose acetate electrophoresis. The presence of bands from HSP aggregates is probably related to the secretion of HSPs during the conditioning of samples at boiling point. FTIR tests revealed that heating a dry organic chicken skin to boiling point leads to the disappearance of a wide amide bands in the 1650–1550cm^{-1} area and leads to the conversion of a band in the 1700–1600 cm^{-1} area, which may be attributed to the intermolecular β-sheet aggregates.

Generally, recording infrared spectra from solutions of pure biochemical compounds using FTIR spectroscopy has been very complicated, but determination of changes in FTIR spectra of liquid samples is fundamental to the discovery of valid biomarkers. Comprehensive analysis of the FTIR spectra in liquid samples is currently under preparation [6]. In this study, only the examples of FTIR spectra were recorded from frozen samples of thermally modified serum. Analysing such samples is complicated; the spectra obtained result in poor signal to noise ratios from liquid body fluids and the strong contribution of water at 1638 cm^{-1}. First, changes of the position of the amide I band were analysed (Table 1, Figure 1). The most diagnostic peaks in the IR spectra have the locations presented in Table 1. However, when increasing the concentration of the solution, the different features of the samples can be seen to systematically evolve. The single band at 1652 cm^{-1} observed in a serum environment modified by the presence of HSPs. The amide I band gradually shifts from 1661 cm^{-1} (Figure 1b) to 1657 cm-1 (Figure 1c) and the amide II band at 1550 cm-1 becomes better defined. A new band around 1580 cm-1 also appears (Figure 1c).

Albumins are the most common serum proteins [6]. Their thermosensitivity and thermo tolerance were the subject of this study concentrating on cellulose acetate membrane electrophoresis. During the inflammatory reaction following heat shock, albumin concentration decreases, and it can even convert to lower-weight

Table 1: The most diagnostic peak (amide I, II and amide III bands) in the IR spectra of the solution of HSA.

liquid samples	Band location (cm^{-1})
a	1681 1661 1643
b	1660 1652 1644
c	1662 1645 1634
d	1663 1648 1634
e	1661 1643 1633
f	1652
g	1657 1644
h	1650 1637
i	1660 1643

(a) the solution of HAS in presence of pure chicken skin;
(b) the solution of HAS in presence of the solution of HAS in presence of pure chicken skin;
(c) the solution of HAS in presence of chicken skin heated to boiling point for 30 s and then incubated in presence of HSA in temperature about boiling point for 30 s;
(d) the solution of HAS in presence of chicken skin heated to boiling point for 35 s and then incubated in presence of HSA in temperature about boiling point for 30 s;
(e) the solution of HAS in presence of chicken skin heated to boiling point for 40 s and then incubated in presence of HSA in temperature about boiling point for 30 s;
(f) untreated concentrated human serum albumin (HSA);
(g) concentrated HSA in presence of chicken skin (HSAS);
(h) concentrated HSA in presence of chicken skin heated to boiling point for 5 s;
(i) concentrated HSA in presence of chicken skin heated to boiling point for 60 s.

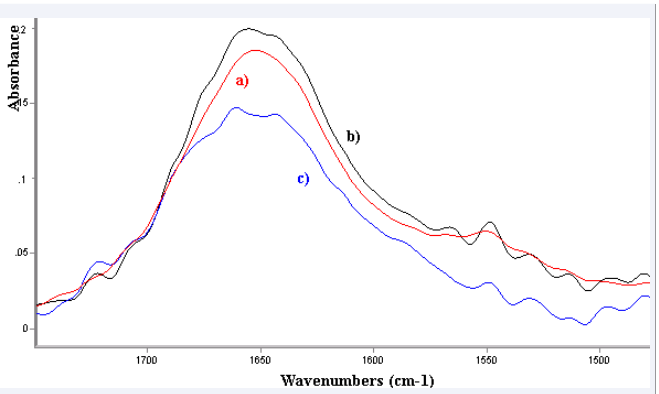

Figure 1 Fragment of the FTIR spectra of liquid samples after freezing: (a) untreated concentrated human serum albumin (HSA); (b) concentrated HSA in presence of chicken skin (HSAS); (c) solution of HSA in presence of chicken skin (HSASS).

proteins, possibly caused by complexes – associates forming in the serum. In our new CAE study [7] (Figure 2), a significant reduction of albumin concentration was observed during heat stress and associates with lower molecular weight were found. The presence of bands from HSP aggregates is probably related to the secretion of HSPs during the conditioning of samples at boiling temperature. Local expression of HSPs in burnt tissue was ealier examined [8-9], which demonstrated increased concentration of HSP32 and HSP70 in the skin following second - and third-degree burns. The

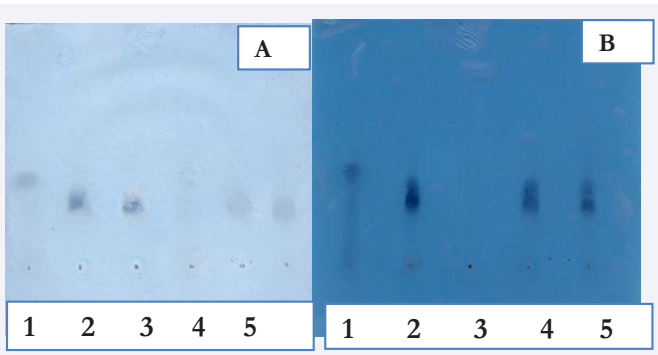

Figure 2 Cellulose acetate membrane electrophoresis. From left to right: (1A) untreated HSA; (2-3A) HSA in presence of skin heated to boiling point for 60s and 120s, and then incubated at boiling point for 30s; (4A) HSA incubated at L-Ascorbic Acid solution; (5A) HSA in presence of skin heated to boiling point for 60s and incubated at L-Ascorbic Acid solution; From left to right: (1B) untreated HSA; (2B) HSA in presence of chicken skin heated to boiling point for 60s and then incubated at boiling point for 60s; (3B) HSA in presence of chicken skin heated to boiling point for 60s and then incubated at boiling point for 60s in presence of hydrogels like H4SiO4 × nH2O (pH 2-3); (4B) HSA in presence of chicken skin heated to boiling point for 60s and then incubated at boiling point for 60s in presence of hydrogels like H4SiO4 × nH2O (pH 6-7); (5B) HSA in presence of chicken skin heated to boiling point for 60s and then incubated at boiling point for 60s in presence of hydrogels like H4SiO4 × nH2O (pH 12-13).

electropherogram of samples in sample example (Figure 2) reveals an additional oligomer band, possibly from HSP32, which undergo atrophy in presence of hydrogels like $H_4SiO_4 \times nH_2O$ or L-Ascorbic Acid solution. Comprehensive analysis of the electropherogram of samples is currently under preparation [7].

CONCLUSION

Aggregates of HSP37 proteins were isolated in this study using cellulose acetate electrophoresis. Determination of changes in FTIR spectra of liquid samples is fundamental to the discovery of valid biomarkers, so the examples of FTIR spectra were recorded from frozen samples of thermally modified serum. Further research will concentrate on finding an effective shielding modifier supporting the process of neutralizing the effects of thermal oxidative stress. In general, it seems that only a comprehensive analysis (CAE, IR and microbiological procedure) can be regarded as an effective biomarker, an attempt to interpret the physicochemical response to heat shock or stress.

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