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S. Y. Lin · R. C. Liang · C. H. Yang · H. S. Hsu
A. T .L. Lin

The effect of vesical outlet obstruction on the protein secondary structure of the mucosa and serosa in rabbit bladder wall

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Abstract The biochemical composition of physiologically moist mucosa and serosa of rabbit bladder before and after bladder outlet obstruction was determined by means of FT-IR spectroscopy with the ATR method and second-derivative analysis. A predominantly β-sheet structure was found in the amide I band for mucosa and serosa before and after obstruction, but the random coil structure increased in both obstructed bladder samples. However, the major β-sheet structure associated with some α-helical structure in the amide II band of mucosa and serosa for non-obstructed bladder changed into a predominantly α-helical structure after bladder obstruction. The obstructed bladder serosa was more pronounced. The amount of glycoproteins doubled in the obstructed bladder serosa, but did not change in the bladder mucosa.

Key words Bladder outlet obstruction · Mucosa · Serosa · Rabbit · FT-IR · ATR · Protein secondary structure

Bladder outlet obstruction is a common clinical problem occurring most often in men with benign prostatic hypertrophy. It has been shown to produce histological and functional mural changes, particularly ingrowth of intercellular collagen and smooth muscle hypertrophy [6, 16, 18, 19]. The bladder wall thickens with outlet obstruction because the smooth muscle mass increases out of proportion to the connective tissue. Obstruction may produce changes in contractile function and passive properties of the rabbit bladder [9]. After obstruction, the emptying ability of the urinary bladder may be reduced, with increasing bladder stiffness. The capacity of stress-relaxation can also diminish with marked fibrous thickening of the serosal layer. Moreover, the observed interstitial fibrosis and change in the smooth muscle cells may also help to account for the functional impairment and disturbed viscoelasticity of the obstructed urinary bladders. Nevertheless, it is still unknown whether these changes, in passive properties or contractility, are related to the secondary conformational structure of bladder wall after obstruction.

Fourier-transform infrared (FT-IR) spectroscopy as a non-destructive approach to the examination of biological materials has provided interesting and useful information on the secondary conformation of intact protein in biological tissues [13]. We have studied the conformational structure of stratum corneum before and after treatment with enhancers, and lens capsules of eyeballs affected by pressure, using a FT-IR spectrometer with attenuated total reflectance (ATR) [10–12]. In the present study we investigated changes in the biochemical composition of rabbit bladder mucosa and serosa after bladder outlet obstruction by means of direct determination using FT-IR spectroscopy with ATR. Second-derivative analysis relative to the original infrared spectra was also carried out.

Materials and methods

Study materials

Male New Zealand white rabbits weighing 2.5–3 kg were used. Normal and obstructed bladders were examined in eight rabbits. The obstruction was achieved by placing a Teflon ring (inner diameter 7 mm) around the urethra just below the bladder neck. Care was taken not to compromise the vesical artery [9]. After obstruction for 2 weeks, the rabbits were killed and the bladders removed. The isolated bladder wall samples were rinsed with neutral isotonic saline solution and wiped carefully with filter paper. Both sides of the moist bladder wall before and after obstruction were directly examined by FT-IR spectrophotometry, in situ [14]. The interstitium of the muscle layer, difficult to dissect completely, will be investigated in the future.

S. Y. Lin (✉)[1] · R. C. Liang
Biopharmaceutics Laboratory, Department of Medical Research,
Veterans General Hospital-Taipei,
Taipei, Republic of China

C. H. Yang · H. S. Hsu · A. T. L. Lin
Division of Urology, Department of Surgery,
Veterans General Hospital-Taipei,
Taipei, Republic of China

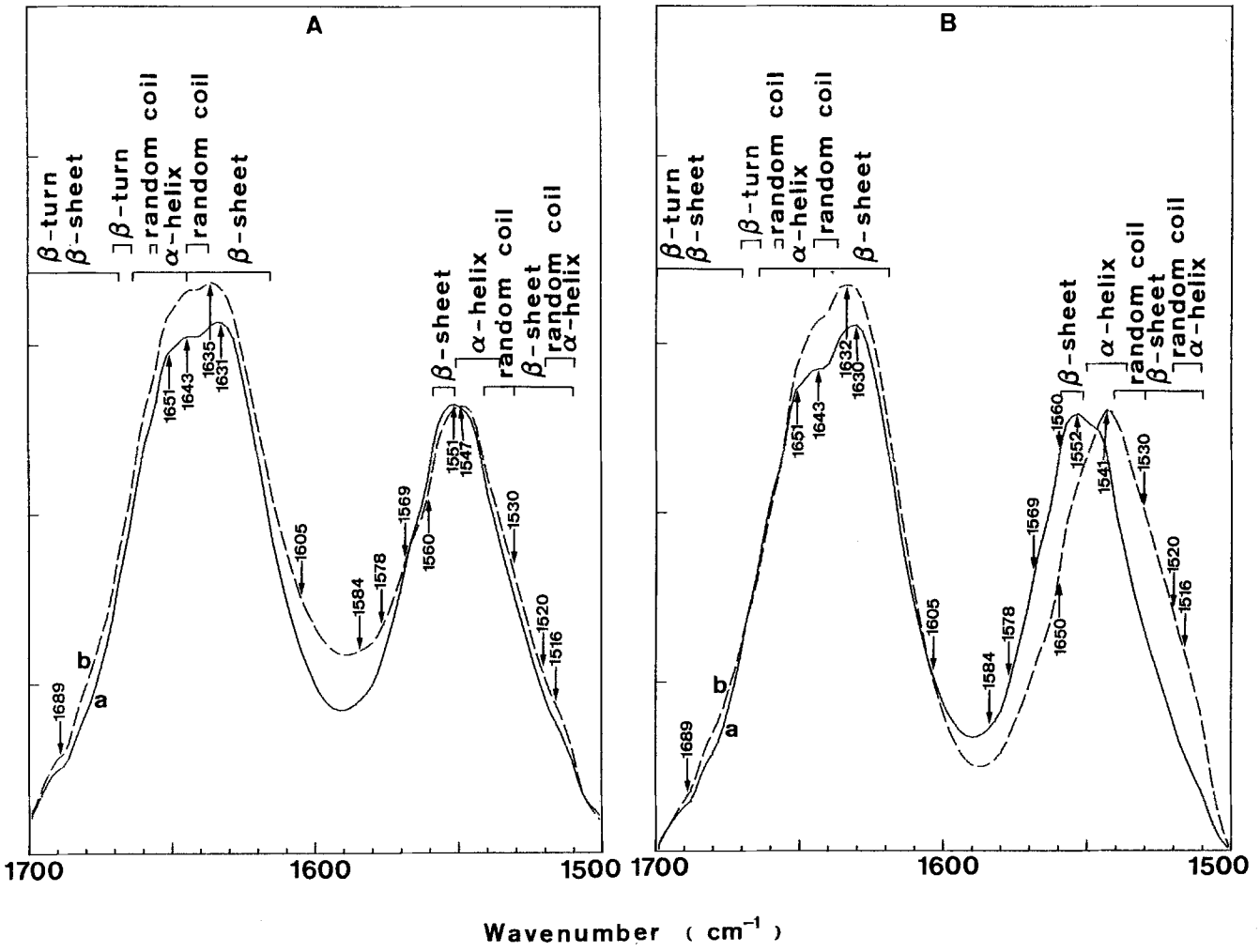

Fig. 1 Fourier-transform infrared absorbance spectra for the mucosa (**A**) and serosa (**B**) of rabbit bladder before (*solid line*) and after (*dotted line*) bladder outlet obstruction

FT-IR determination

The isolated bladder wall samples were placed directly on ATR prisms, and IR spectra were obtained using a FT-IR spectrophotometer equipped with an MCT detector and a zinc selenide ATR prism (Micro-FT-IR 200, Jasco, Japan) [14]. The spectra were taken at 4 cm^{-1} resolution, and generally 200 scans were accumulated to get a reasonable signal-noise ratio. The spectra presented in this study were all difference spectra between the spectra of samples and those of water, which were calculated by normalizing the intensity of a band near 2120 cm^{-1} due to water [11–12]. Detailed analysis of these spectra was achieved using a second-derivative procedure.

Results are given as mean ± SD, and the numbers of determinations are given in parentheses. Statistical comparisons were made using analysis of variance, assuming $P < 0.05$ as significant.

Results

The amide I and II bands are sensitive to the secondary structure of polypeptides and proteins, and are most useful for the analysis of protein secondary conformational structure [4]. The FT-IR spectra of the mucosa and serosa of the rabbit bladder before and after obstruction are shown in Fig. 1. The estimated composition of protein secondary structure for the characteristic amide I and II bands are also indicated. The second-derivative spectra also support the information of the side chains and the secondary conformational structure of protein. Before bladder outlet obstruction, the maximum frequency of amide I for bladder mucosa centered near 1631 cm^{-1} assigned to β-sheet structure, with a shoulder near 1643 cm^{-1} assigned to random coil structure. The maximum absorption peak in the amide II region was located at 1551 cm^{-1}, assigned to the β-sheet structure with some α-helical structure (Fig. 1A,a) [1]. After obstruction for 2 weeks, the above two peaks shifted to 1635 and 1547 cm^{-1}, respectively (Fig. 1A,b). The band at 1635 cm^{-1} was still assigned to β-sheet structure, but the peak at 1547 cm^{-1} was assigned to the predominantly α-helix structure. The bladder outlet obstruction modified the amide II conformation of bladder mucosa to a predominantly α-helical structure. Moreover, greater contributions of random coil structure at 1643 cm^{-1} and of α-helical structure at 1651 cm^{-1} were found for the obstructed bladder mucosa due to the decreased peak intensity.

Table 1 Peak area ratio (mean ±SD) of infrared spectra for the mucosa and serosa of rabbit bladder before and after bladder outlet obstruction

Samples	Peak area ratio				
	Amide I / Amide II	Scissoring / Amide II	COO^- / Amide II	Amide III / Amide II	Glycoprotein / Amide II
Normal mucosa ($n = 8$	1.495 ± 0.187	0.112 ± 0.007	0.116 ± 0.010	0.353 ± 0.026	0.252 ± 0.053
Obstructed mucosa ($n = 8$)	1.799 ± 0.148	0.098 ± 0.008	0.131 ± 0.021	0.283 ± 0.026	0.261 ± 0.041
ANOVA test	*NS*	*NS*	*NS*	*$P < 0.05$*	*NS*
Normal serosa ($n = 8$)	1.799 ± 0.247	0.131 ± 0.009	0.098 ± 0.020	0.380 ± 0.022	0.175 ± 0.039
Obstructed serosa ($n = 8$)	1.491 ± 0.197	0.111 ± 0.012	0.125 ± 0.016	0.316 ± 0.027	0.336 ± 0.076
ANOVA test	*NS*	*NS*	*NS*	*$P < 0.05$*	*$P < 0.05$*

(amide I 1700–1590 cm^{-1}, amide II 1590–1480 cm^{-1}, scissoring band 1480–1430 cm^{-1}, carboxylate 1430–1350 cm^{-1}, amide III 1350–1180 cm^{-1}, glycoprotein 1180–980 cm^{-1})

Figure 1B clearly indicates that the IR spectra and conformational structure of amide I band for the obstructed bladder serosa were similar to those of the non-obstructed bladder serosa. Therefore, the conformation should mainly be β-sheet structure (1630 and 1632 cm^{-1}). The amide I component at 1643 and 1656–1658 cm^{-1} assigned to random coil structure also made a major structural contribution to the obstructed bladder serosa [8]. The most significant difference between obstructed and non-obstructed bladder serosa was found in the amide II band. Apparently, the entire amide II band for the non-obstructed bladder serosa shifted about 11 cm^{-1} to a lower wavenumber after obstruction for 2 weeks. The amide II peak at 1552 cm^{-1}, assigned to β-sheet structure with some α-helical structure for non-obstructed bladder serosa, transformed to a predominantly α-helical structure for the obstructed bladder serosa with the amide II peak at 1541 cm^{-1}. In addition, the increased peak intensity for amide II band shoulder at 1520 and 1530–1540 cm^{-1} strongly suggested the presence of some random coil structure for the obstructed bladder serosa [8]. Thus, urinary bladder obstruction can considerably modify the protein secondary structure of bladder mucosa and serosa in rabbit, especially when the obstructed bladder serosa becomes more pronounced. The amide II component at 1516 cm^{-1} for the obstructed bladder serosa may be attributed to the α-helical and random coil structure, although it can also be assigned to tyrosine side chains [5].

Discussion

It is well known that histological and functional changes may occur when the adult bladder is subjected to chronic outlet obstruction. Histological studies of obstructed bladders usually indicate smooth muscle hypertrophy, greater amount but a lesser concentration of collagen and an increase in elastin [3, 18]. Collagen and elastin are the predominant components of the bladder wall that influence bladder function. Collagen provides tensile strength, but an overaccumulation may inhibit bladder contractility [6]. Elastin provides tissue elasticity and should help compliance [17]. Collagen deposition and increased fibrous tissue are found throughout the layers of the bladder wall after obstruction. Considerable interstitial fibrosis thickening in the serosal layer [9] becomes the major cause of increased stiffness after obstruction. This stiffness might possibly modify the secondary structure of protein in the obstructed bladder wall, decreasing its contractility and passive function.

IR spectra can provide structural information about group functionality, bond type and strength, and environmental influences at the molecular level [4, 8]. From the second-derivative spectra, the absorption peaks between the amide I and II peak components may be attributed to amino acid side chains [2]; peaks at 1604, 1588, and 1578 cm^{-1} may be associated with arginine residues; peaks at 1568 and 1558 cm^{-1} with glutamic acid residues (Fig. 1). Some contribution of aspartic acid residues may be present in the peak at 1586 cm^{-1}. It is obvious that arginine residues and/or aspartic acid residues in the obstructed bladder mucosa had higher peak intensity than those of the non-obstructed bladder mucosa. However, the obstructed bladder serosa apparently had lower peak intensities of arginine residues and/or aspartic acid residues, and glutamic acid residues, than the non-obstructed bladder serosa. Although the obstructed organ had richer elastin in polar amino acids than the normal bladder [3], the significance of these changes is still unknown. Further study seems warranted.

The characteristic range of the secondary structure for each band is listed in Table 1. The peak area ratio of each band to amide II is also tabulated. We found no significant difference in the peak area ratio of amide I/amide II, scissoring band/amide II or carboxylate group/amide II between non-obstructed and obstructed groups, whether mucosa or serosa ($P > 0.05$). On the other hand, the peak area ratio for amide III/amide II and glycoprotein group/amide II was different between non-obstructed bladder and obstructed bladder. The secondary structural components of the amide III were assigned to α-helical structure

near 1300 and 1317 cm^{-1} and to random coil structure near 1242 cm^{-1} [5]. The band near 1282 cm^{-1} was also assigned to helix or disordered structure. The significantly decreased peak area ratio of amide III/amide II for bladder mucosa and bladder serosa after obstruction ($P < 0.05$) suggested the possibility of change in the secondary structure of α-helical and randon coil structures. The glycoprotein layer on the mucosal surface of the bladder has been proposed as the most important defence mechanism in the urinary tract [15]. It was therefore very interesting to find that glycoprotein/amide II peak area ratio of bladder mucosa was similar between non-obstructed bladder and obstructed bladder ($P > 0.05$). The bladder outlet obstruction seemed not to change the amount of glycoprotein. Thus, the host defence mechanism of glycoprotein in shielding the bladder wall from pathogens seems to be of no influence. However, the glycoprotein /amide II peak area ratio for bladder serosa increased about twofold after obstruction ($P < 0.05$), possibly because of an increase in intercellular collagen [3].

Conclusions

It is interesting that protein secondary structure of bladder mucosa and serosa was considerably modified after mild outlet obstruction of the urinary bladder for 2 weeks, the obstructed bladder serosa being the more pronounced. The possibility that this conformational modification changes bladder function merits investigation. The contribution of amino acids to the protein conformation of bladder mucosa and serosa seemed to be effected by obstruction. The glycoprotein content of bladder serosa increased significantly after obstruction, but bladder mucosa was not influenced in this way.

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