CHAPTER IV
RESULTS

4.1 Analysis of proteome profile in renal cortex and medulla related with K status in subjects living in northeastern Thailand

We performed renal cortex and medulla proteome by gel-based method to define the differentially expressed proteins in left upper quadrant of the renal obtained from vehicle-accident cadaveric males of KD and NKD subjects. The renal tissue proteins of both cortex and medulla were separated by 2D-PAGE, followed by Coomassie Brilliant Blue staining. Image Master 2D Platinum software was employed to compare the protein spot pattern in all 2-D gels (6 gels in each group; totally 12 gels were analyzed). To test for differences between the two groups, an unpaired *t*-test was used, and the *p*-values < 0.05 were considered statistically significant. These altered proteins were then subjected to mass spectrometric protein identification with the use of Q-TOF MS and MS/MS analyses. The identified proteins were classified based on their major roles in biological processes, including metabolic enzyme, signaling proteins, RNA metabolism, cell division, DNA repair, immune responses, cytoskeletal protein, transporter protein, small GTP-binding protein, novel protein, Rho protein signal transduction, transcription regulation. We also performed database search using the information appeared in the well-established protein data base including NCBI (http://www.ncbi.nlm.nih.gov/), Swiss-Prot/TrEMBL (http://expasy.org/) and Human Cyc (http://humancyc.org/) to obtain additional details for their function. Their synonyms and details for their functions, as well as metabolic pathways involved, are summarized in Table 12 and 13. This study is the first time that proteomics has been applied to characterize the altered renal cortex and medulla proteome during K depletion in human.

4.1.1 Alteredant proteome in KD renal cortex

The results of 2 DE image of renal cortex proteome profile revealed that up to 250 protein spots were visualized in each 2 DE gel (Figure 8). Quantitative intensity analysis and statistics revealed significantly differential expression of ten protein spots (labeled with numbers in Figure 8), including nine up-regulated and one down-regulated spots. The degrees of alterations range from 1.34-1.89 fold increases and 0.70 fold decrease (Table 6). These altered proteins were then subjected to mass spectrometric protein identification. With the use of Q-TOF MS and MS/MS analyses, 10 of 10 altered proteins were successfully identified. Their identities, identifiers, PMF scores, MS/MS ions scores, percentages of sequence coverage (%Cov), number of matched peptides, and intensity data summarized in Table 7.

Most of the altered proteins identified in this study were metabolic enzyme (glutathione S-tranferase P, acyl-coA thioesterase 9), signaling proteins (SPARC-like 1, protein tyrosine kinase 2-beta isoform 3), RNA metabolism (ATP-dependent RNA helicase DDX60), cell division (spindle and kinetochore-associated protein 1), DNA repair (general transcription factor IIH subunit 1), and immune responses (immunoglobulin heavy chain variable region, T cell receptor variable chain). In this study, we evaluated the CV of selected protein spots within group (NKD, KD). The summarized data of %CV of these protein spots were shown in Table 10.

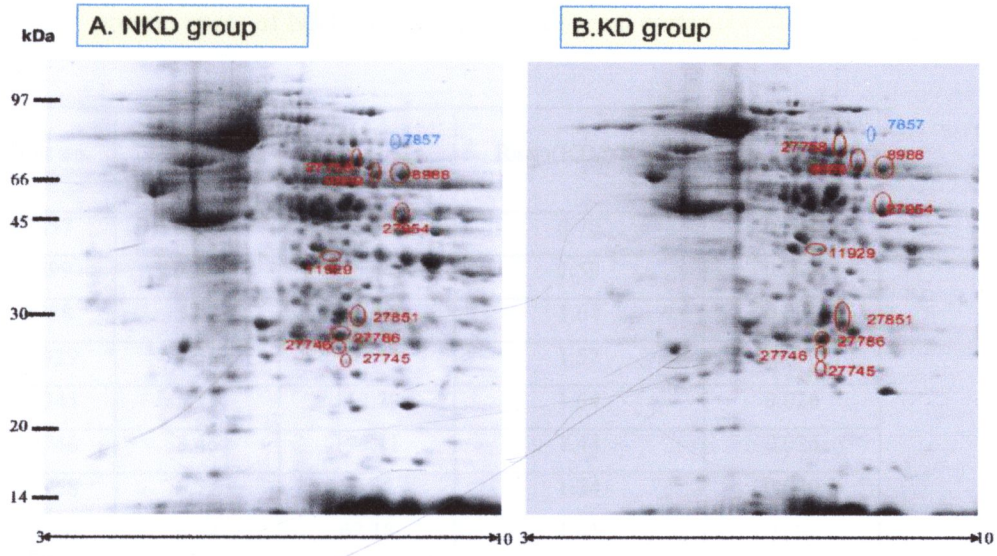

Figure 8 Proteome map of differentially expressed proteins of NKD (A) and KD (B) groups. Equal amount of total protein (200 μg) extracted from renal cortex tissues (n=6 from different subjects in each group) was loaded on gel and resolved by 2-DE. Separated proteins were separated by Coomassie Brilliant Blue R-250 stain. Quantitative intensity analysis was performed and these spots with significant differences between the two groups by two-sample test and were labeled (red and blue circled spot donate the increased and decreased proteins, respectively).
Detailed protein expression data as shown in Table 6 and 7

Table 6 Mean of volume intensity of the nine up-regulated and one down-regulated protein spots of NKD and KD groups of renal cortex

Spot no.	p*I*	Mw (kDa)	Ratio(KD/NKD)	*p*-values
7857	4.72	75.85	0.70	0.021
8899	5.82	16.44	1.59	0.018
8988	5.82	16.44	1.67	0.001
11929	4.55	2.68	1.52	0.036
27745	5.77	112.29	1.64	0.026
27746	8.46	199.11	1.44	0.025
27758	6.77	29.62	1.34	0.030
27786	8.74	62.18	1.41	0.002
27851	6.89	23.83	1.36	0.023
27954	8.80	50.75	1.89	0.002

Table 7 Summary of differentially expressed proteins of renal cortex between NKD and KD groups

Spot	Protein	NCBI ID	Identified by	Identification scores (MS, MS/MS)	%Cov (MS, MS/MS)	No. of matched peptides (MS, MS/MS)
7857	SPARC-like 1	gi|197101799	MS	95, NA	26, NA	13, NA
8899	Immunoglobulin heavy chain variable region	gi|37777992	MS	72, NA	59, NA	7, NA
8988	Immunoglobulin heavy chain variable region	gi|37777992	MS	78, NA	59, NA	7, NA
11929	T cell receptor variable beta chain	gi|245769	MS/ MS	NA, 32	NA, 84	NA, 1
27745	Protein-tyrosine kinase 2-beta isoform 3	gi|241982783	MS	70, NA	18, NA	11, NA
27746	ATP-dependent RNA helicase DDX60	gi|194208330	MS	72, NA	13, NA	15, NA
27758	Spindle and kinetochore-associated protein 1	gi|229891748	MS	76, NA	36, NA	8, NA
27786	General transcription factor IIH subunit 1	gi|194213897	MS	72, NA	23, NA	11, NA
27851	Glutathione S-transferase P	gi|29135329	MS	82, NA	40, NA	7, NA
27954	Acyl-CoA thioesterase 9	gi|62078649	MS	72, NA	28, NA	9, NA

4.1.2 Alteredant proteome in KD renal medulla

The results of 2 DE image of renal medulla proteome profile revealed that up to 200 protein spots were visualized in each 2 DE gel (Figure 9). Quantitative intensity analysis and statistics revealed significantly differential expression of eleven protein spots (labeled with numbers in Figure 9), including four up-regulated and seven down-regulated spots. The degrees of alterations range from 1.21-1.55 fold increases and 0.61-0.85 fold decrease (Table 8). These altered proteins were then subjected to mass spectrometric protein identification. With the use of Q-TOF MS and MS/MS analyses, 11 of 11 altered proteins were successfully identified. Their identities, identifiers, PMF scores, MS/MS ions scores, percentages of sequence coverage (%Cov), number of matched peptides, and intensity data summarized in Table 9.

Most of the altered proteins identified in this study were metabolic enzyme (catalase, cabonic anhydrase 1, enoyl-Coenzyme A, hydratase/3-hydroxyacyl-Coenzyme A dehydrogenase isoform 1), cytoskeletal protein (actin-gamma1), transporter proteins (ADP.ATP translocase), small GTP-binding protein (Rab11 family-interacting protein 2), immune responses (T-cell receptor beta chain, immunoglobulin heavy chain complementarity-determining region 3), novel protein (riken cDNA 1700001E04), Rho protein signal transduction (guanine nucleotide exchanger factor GEFT isoforms 3), transcription regulation (enhancer of polycomb homolog 1 isoforms 2). The summarized data of %CV of these protein spots were shown in Table 11.

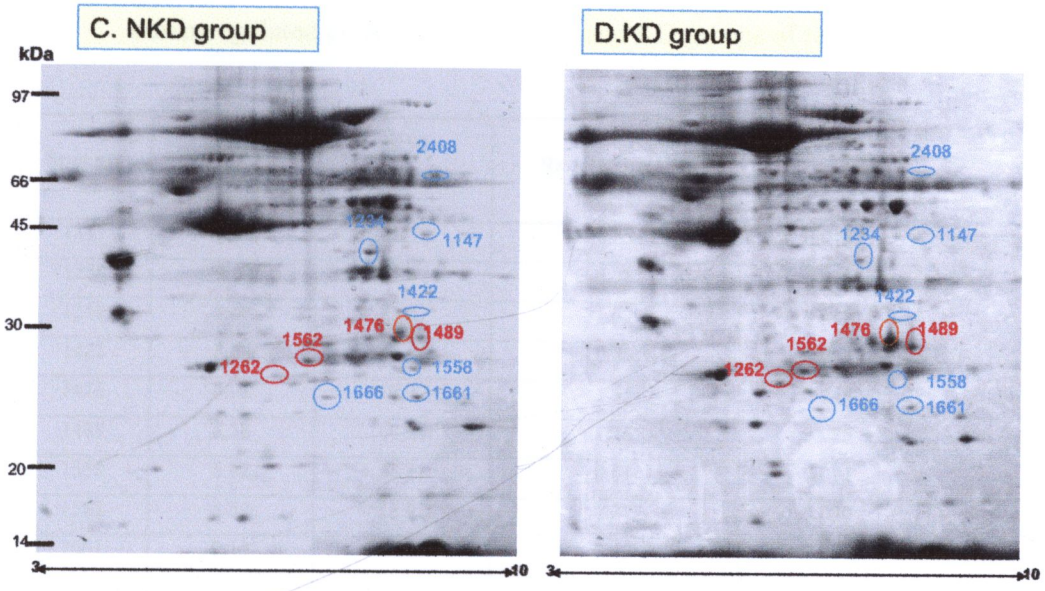

Figure 9 Proteome map of differentially expressed proteins of NKD (C) and KD (D) groups. Equal amount of total protein (200 μg) extracted from renal medulla tissues (n=6 from different subjects in each group) was loaded on gel and resolved by 2-DE. Separated proteins were separated by Coomassie Brilliant Blue R-250 stain. Quantitative intensity analysis was performed and these spots with significant differences between the two groups by two-sample test and were labeled (red and blue circled spot donate the increased and decreased proteins, respectively).
Detailed protein expression data as shown in Table 8 and 9

Table 8 Mean of volume intensity of the four up-regulated and seven down-regulated protein spots of NKD and KD groups of renal medulla

Spot no.	p*I*	MW (kDa)	Ratio(KD/NKD)	*p*-values
1147	7.33	61.51	0.80	0.015
1234	9.04	17.47	0.78	0.025
1262	9.68	28.27	1.32	0.018
1422	8.88	34.75	0.85	0.025
1476	6.92	27.92	1.55	0.044
1489	5.58	68.67	1.26	0.025
1558	8.82	85.31	0.61	0.013
1562	6.59	28.91	1.21	0.049
1661	9.13	76.22	0.65	0.026
1666	9.33	58.36	0.79	0.011
2408	8.20	2.57	0.66	0.017

Table 9 Summary of differentially expressed proteins of renal medulla between NKD and KD groups

Spot no.	Protein	NCBI ID	Identified by	Identification scores (MS, MS/MS)	%Cov (MS, MS/MS)	No.of matched peptides (MS, MS/MS)
1147	Catalase	gi|194217817	MS, MS/ MS	65, 70	29, 5	11,2
1234	Actin, gamma 1	gi|169160859	MS/MS	NA, 49	NA, 10	NA, 1
1262	ADP.ATP translocase	gi|339721	MS/MS	NA, 50	NA, 6	NA, 1
1422	T-cell receptor beta chain	gi|32892048	MS	52, NA	25, NA	8, NA
1476	Riken cDNA 1700001E04	gi|293362413	MS	76, NA	46, NA	7, NA
1489	Guanine nucleotide exchange factor GEFT isoform 3	gi|162287076	MS	74, NA	19, NA	9, NA
1558	Enhancer of polycomb homolog 1 isoform 2	gi|119905288	MS	62, NA	17, NA	9, NA
1562	Carbonic anhydrase 1	gi|4502517	MS, MS/ MS	119, 102	57, 12	11, 2
1661	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase isoform 1	gi|114590838	MS	73, NA	25, NA	9, NA
1666	Rab11 family-interacting protein 2	gi|7662394	MS	85 NA	30, NA	12, NA
2408	Immunoglobulin heavy chain complementarity-determining region 3	gi|29568315	MS/ MS	NA, 32	NA, 87	NA, 1

Table 10 %CV of significantly altered protein spots in renal cortex

Spot no.	%CV	
	NKD	KD
7857	23.86%	12%
27954	16.85%	8.33%
27758	15.97%	13.40%
27851	6.31%	10.63%
27745	13.67%	15.56%
27746	8.53%	13.78%
11929	6.41%	15.21%
8899	13.18%	12.81%
27786	9.09%	3.59%
8988	17.89%	10.11%

Table 11 %CV of significantly altered protein spots in renal medulla

Spot no.	%CV	
	NKD	KD
2408	23.87%	19.47%
1234	7.28%	5.77%
1147	20%	15.52%
1422	10.28%	18.68%
1666	13.95%	23.53%
1661	9.77%	12.64%
1558	16.41%	10.83%
1262	18.37%	24.03%
1562	18.32%	17.42%
1476	14.58%	13.85%
1489	15.68%	21.21%